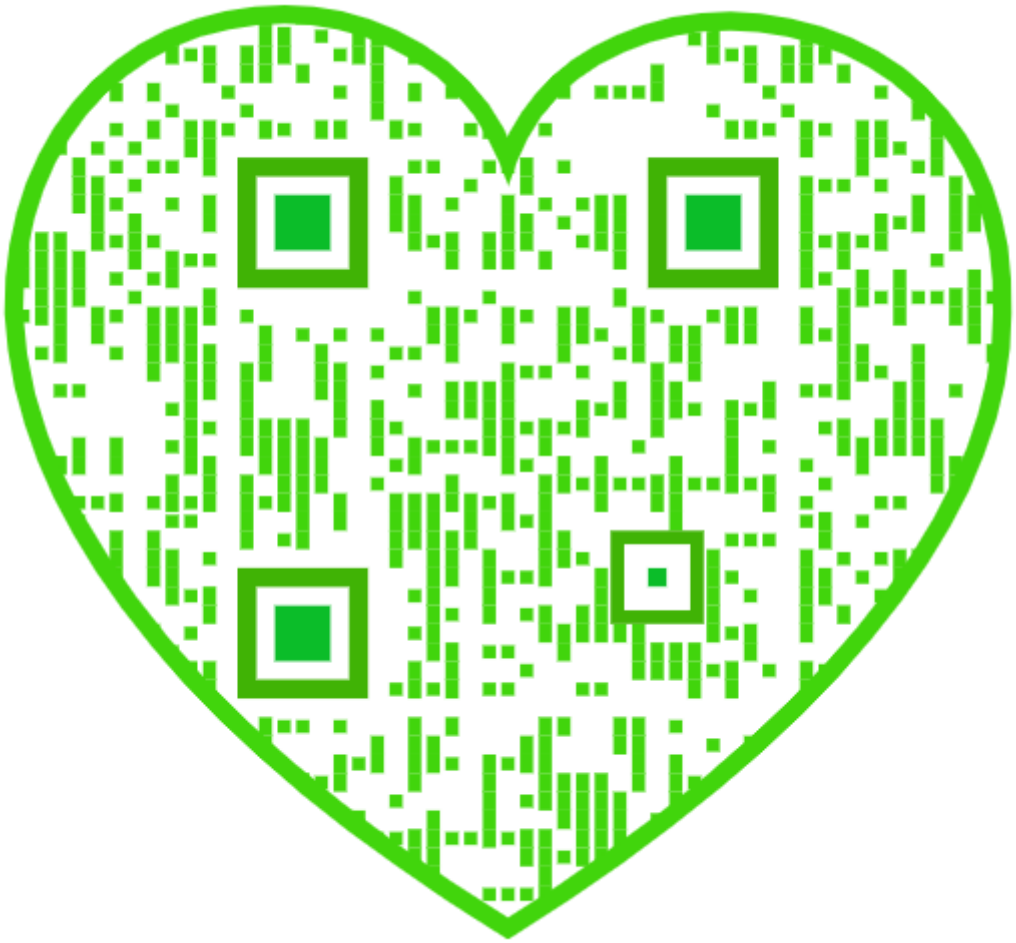

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ASSISTED BY OTHER BOTANISTS

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The Development of the Seed in the Scitamineae.

BY

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With Plates I-IV.

THE following pages contain the results of studies begun in the Botanical Institute of the University at Bonn and continued in America, chiefly in the Biological Laboratory of the Johns Hopkins University. The line of research was suggested by observations made incidentally on *Canna*, the study of which genus was begun with a very different purpose. As the material proved worthless for the work originally planned, but showed features of unexpected interest in connexion with the development of the seed from the ovule, the essential details of this process were followed out. The striking peculiarities shown by *Canna* made it seem desirable to compare other Scitamineae, especially since the plants of this order are so intimately related among themselves, yet occupy a position comparatively isolated with respect to other Monocotyledonous families. Although not so many forms have been examined as could be wished, it does not seem necessary

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to delay longer the publication of the results already reached, since it is difficult to obtain suitable material of these almost exclusively tropical plants.

The materials studied have been preserved in alcohol and have been drawn from various sources, as follows: *Canna indica*, L., from the beds, and *Strelitzia Reginae*, Ait., from the greenhouses of the Botanic Garden at Bonn; *Musa* sp. and *Heliconia psittacorum*, L. fil., and *H. Bihai*, L., from Mr. E. Campbell, lately superintendent of the Botanic Gardens at Castleton, Jamaica, through the courtesy of the Director of Public Gardens and Plantations, Mr. Wm. Fawcett, F.L.S.; *Costus* sp. and *C. speciosus*, Sm., *Alpinia mutica*, Roxb., and *Elettaria* sp., from the Botanic Gardens at Buitenzorg, Java, by the generosity of the Director, Dr. M. Treub; *Amomum elongatum* (Teijsm. et Binn.), *Alpinia alata*, A. Dietr., and *Phrynium capitatum*, Willd., brought by him from Java, most kindly placed at my disposal by Prof. A. F. W. Schimper, of Bonn; *Thalia dealbata*, Fraser, *Calathea densa*, Regel, and *Globba bulbifera*, Roxb., collected by myself at the Royal Gardens, Kew, by permission of the Director and under the genial guidance of the Curator, George Nicholson, Esq., F.L.S. To each of the gentlemen above named I am under deep obligation for the willing assistance which has made these studies possible. And I owe to Prof. Eduard Strasburger the freest use of the Botanic Garden and of his library, in addition to the inestimable advantage of his constant advice and cordial interest during my work in Bonn.

In the arrangement of Monocotyledonous plants by Bentham and Hooker, the Scitamineae are treated as a single 'natural order,' while the arrangement of the modern German systematists makes of these plants a series co-ordinate with the Liliiflorae, Glumiflorae, and the rest. And indeed the marked variations which the members of the group show among themselves makes the latter treatment seem preferable. These plants agree with most Monocotyledons in their typically tricarpellary gynaeceum and diplostemonous androecium. Chiefly on the basis of the variations in these parts, the

group has been divided by German authors into three or four families, corresponding to the orders of Bentham and Hooker, while the English writers regard these divisions as of sub-ordinal rank. Most recent writers are disposed to make four sub-divisions, and, as the following pages present additional arguments for this view, the Scitamineae will be here regarded as comprising four distinct groups worthy of family rank.

In the Musaceae the androecium comprises five fertile stamens, with sometimes a small staminodium in place of the sixth. The more or less completely suppressed stamen is that member of the inner whorl which is opposite the odd petal. In all the other families, on the other hand, this is the only functional stamen. The two other members of the inner whorl are fused in the Zingiberaceae to form the large petaloid 'labellum,' while in the Cannaceae and Marantaceae they remain petaloid and separate. In the Cannaceae one forms a labellum, its homologue in the Marantaceae being thin and crumpled and known as the 'hood.' The other inner member forms the flat 'wing' of the Cannaceae, but is thickened and known as the 'callus-leaf' in the Marantaceae. The outer whorl may be represented by one or more petaloid staminodia, or may be entirely suppressed, in these three families. The fertile stamen of the Zingiberaceae is complete; but in the other two families only one half or lobe produces pollen, while the other half is represented by a petaloid expansion.

The inferior ovary of all the Scitamineae, except a small group of the Zingiberaceae, is trilocular, and most commonly contains numerous anatropous ovules borne on axile placentas. But in the genus *Heliconia* of the Musaceae, and in all the Marantaceae, each loculus contains only a single ovule rising from its base, while in some of the genera of the latter family only one loculus is fertile. Except in the cases just referred to, the fruit is a several- or many-seeded capsule or berry; in these it is dry, imperfectly dehiscent, and three-seeded, except in the one-seeded Marantaceae. As will be seen, the structure of the seeds differs much in the various families; but the presence of some structure comprised under the indefinite term

'aril,' though not universal throughout the Scitamineae, is yet so general that the group has also been called '*Arillatae.*'

The changes undergone by the ovule from its first appearance to the time of fertilization, and the subsequent growth and differentiation which takes place normally in response to the stimulus of sexual union, have been studied in detail in many seeds, so that the features of seed-development in general are well known. But observations of this sort relating to the group under consideration are few and fragmentary. They have been made chiefly on species of *Canna*, which is the only Scitamineous genus whose members thrive and perfect seeds in the summer climate of our temperate latitudes. Some of the interesting details of seed-development in this genus have already been noted, but chiefly incompletely or incidentally, so that they have remained little noticed. As already intimated, the independent discovery of these striking features has led to the present comparative studies, in the hope that some light might be thrown on the phylogeny of the group and the origin of the peculiarities of *Canna*. Although the result is less satisfactory than could be wished in this respect, certain features are shown to be probably common to the Scitamineae, and certain interesting phenomena show how widely the details of seed-development may vary within the limits of a family.

The work has been done chiefly by freehand sectioning. Since the cell-contents were often not important for the question in hand, rather thick sections could be cleared with Javelle water. Indeed, the relations of tissues could often be better made out on such sections than from thinner ones. For special points microtome-sections were necessary, but as a rule they have offered no advantages over those more easily prepared.

Though *Canna* proves to be the most aberrant in its seed-development of all the genera examined, yet, since it was first and most exhaustively studied, its development may be first described. This genus contains, according to the estimates of different monographers, from thirty to sixty

species, which are natives of tropical America. The published figures of the few species whose ovules and seeds have been studied afford ground for the expectation that the development will prove closely similar in all. The species here described is believed to be the true *C. indica*, L. The members of this genus constitute alone the family

CANNACEAE.

The general structure of the ripe seed of *Canna* was studied and more or less accurately figured by Gaertner (1788), Mirbel ('10, '15), and Richard ('11), but we owe the first account of its real character to the founder of developmental morphology, Schleiden. Some of the chief features in the development of the seed were pointed out by him ('39), though they seem since to have been lost sight of. Since his time only fragmentary notes on the genus have appeared, with the exception of Hegelmaier's account ('74) of the development of the embryo.

The ovules make their appearance in each loculus as two vertical rows of outgrowths from the placenta. Each has a distinct epidermal layer, and very early a sub-epidermal cell becomes distinguishable by its size as the archesporial cell. This soon divides into an apical and a sub-apical cell. The former was found in all cases examined (Fig. 1), though Guignard ('82) states that this is not always true, but the number of anticlinal walls by which it is cut to form the tapetal layer seems to vary. Indeed it soon becomes impossible to distinguish between the cells of this layer and adjoining ones of the nucellus. The sub-apical cell, or embryo-sac mother-cell, divides twice, thus producing a row of three cells (Figs. 2, 3). As in other plants, it is the lower cell of the row which forms the embryo-sac.

The development of the ovule proceeds in normal fashion. The outgrowth soon begins to turn upon itself and assume its anatropous form. At about the time of the division of the archesporial cell, the limit of chalaza and nucellus is indicated by the formation of periclinal walls in certain cells of the

epidermal layer (*i. i.*, Fig. 1), which usually form a double row encircling the base of the nucellus. The growth thus begun is continued so that a ridge of tissue results, which finally encloses the whole nucellus except at the micropyle, forming the inner integument of the ovule (*i. i.*, Figs. 2, 3, 4). This coat is usually, as in all the Scitamineae studied, two cells in thickness. It begins to be formed slightly earlier on the outer side of the ovule than on the side turned toward the funiculus (Fig. 1). As its development progresses, and when it has reached perhaps half of the height of the nucellus, there arises in the same manner from the epidermal cells just at its base the outer integument. This has a thickness of several cell-layers, as in all the Scitamineae examined, and, at the time of fertilization is of about the same height as the inner integument, so that the long and narrow micropyle is formed wholly by the latter (Fig. 4). The outer integument is interrupted by and fused with the raphe as in other anatropous ovules. Meanwhile the vascular bundle has developed in the funiculus, and downward to the chalaza of the ovule. The embryo-sac at first lengthens slightly with the growth of the nucellus, and then rapidly broadens at its micropylar end, destroying the tissue lying between itself and the nucellar epidermis, and acquiring the clavate form which it has at the time of fertilization (Fig. 4). At this time, however, not all of the nucellar tissue is suppressed; but the sac is separated from the nucellar epidermis by one or more cell-layers, except at its apex. The nucellar and chalazal portions of the ovule are now about equal, and the lower end of the embryo-sac has already penetrated into the chalaza. The fusion of the polar nuclei to form the endosperm-nucleus (*esp. n.*, Fig. 4) and the developed egg-apparatus (*egg*) have been observed, but the antipodal cells were not well seen. In view, however, of Guignard's observations ('82) of the usual phenomena within the embryo-sac of *Canna*, it was not thought necessary to pursue the subject in detail. The mature ovule is so far turned upon itself that its micropyle lies almost in contact with the funiculus, and the course of the pollen-tube is neces-

sarily a sinuous one. Yet this appears to be no practical difficulty in the way of its performing its office. After it has entered the micropyle and penetrated the nucellar epidermis, and fertilization has taken place, those changes begin which constitute the

Development of the seed. After a general comparison of this process with what occurs in other seeds, we may proceed to a detailed discussion of the fate of each part of the ovule. It may be useful to recall that ordinarily the seed-coat is developed from the integument or integuments of the ovule, while the body of the seed is composed of tissues formed within the embryo-sac or of these and nucellar tissue, the chalaza playing an inconspicuous part. In *Canna* we have seen that about half of the ovule consists of chalazal tissue at the time of fertilization, and we shall find the proportion steadily increasing. This interpretation is based on the current definition of the chalaza as 'the transverse zone from which the single or the two true integuments spring[1],' and of the nucellus as 'der oberhalb des inneren Integumentes liegende Theil des Eichens[2].' Such a distinction is, at best, more or less arbitrary, but that here indicated seems the most natural as well as the most simple.

At the time of fertilization the ovule is about ·5 mm. in length from micropyle to base. By the time it reaches a length of ·8 mm., the micropyle has become quite closed, the base of the embryo-sac has broadened so that it is even wider than the upper end, and there begins to appear a sharp constriction of the funiculus penetrating from its outer side at about the level of the mouth of the micropyle (*c*, Fig. 5). Below this constriction is a distinct lip which pushes rapidly upward and inward as the constriction encroaches upon the vascular bundle (*c*, Fig. 6). When the length of the young seed has reached 2 mm., this lip has nearly met the lip formed by the free end of the outer integument of the other side, and there protrudes from between them the remnant of

[1] Sachs' Text-Book, second Engl. ed., p. 571.
[2] Luerssen's Handb. d. med.-pharm. Bot., Bd. II, p. 258.

the funiculus (*f*, Fig. 7). From this time the connexion of the seed with the placenta is very slight, mechanically; but the vascular bundle must remain functional for a long time, in order to furnish the material for its extensive further growth to the seed. We shall see that these lips never fuse at the point of entrance of the bundle, but that there remains here in the ripe seed a small break in the seed-coat which will be called the *germinal slit*, and which represents, as is evident, the combined micropyle and hilum of the ovule (*g. s.*, Fig. 8). Meantime the greater proportion of growth has taken place in the chalazal region, and this continues to be true for some time, as may be seen from the following table, which gives average figures for several stages up to the full-grown seed:—

Length of seed . .	·80 mm.	2·25 mm.	4·50 mm.	9·50 mm.
Nucellar part . .	·38 „	·65 „	·85 „	1·95 „
Chalazal part . .	·42 „	1·60 „	3·65 „	7·55 „
Per cent. of growth of whole seed	180 %	100 %	111 %	
„ „ of nucellar part	71 %	31 %	129 %	
„ „ of chalazal „	281 %	128 %	107 %	

During this growth the embryo-sac extends downward as a broad irregular cavity more or less abundantly lined with dead and ragged cell-remains; as its full size is reached, it gradually becomes more regular in form and smoother in outline. When the young seed is about 2 mm. long, the upper end of the embryo-sac abruptly expands by the resorption of the tissue lying between it and the inner integument; that is, of practically all the nucellar tissue. This gives the sac a very peculiar form, that of a club-shaped body, broadest at the base, connected by a narrow neck with a broadly conical head (*e. s.*, Fig. 7). The tissue surrounding the neck soon dies and becomes resorbed, so that the neck itself is at last only a slightly narrowed region between head and body (*e. s.*, Fig. 8). Hegelmaier states ('74) that a chalazal cavity is formed independently of the embryo-sac, and becomes united with it later by the breaking down of the intervening tissue. As to this, it can only be said that

the evidence of the accompanying figures (Figs. 5–8) seems conclusive that such is not the case; and I am fully convinced after careful study of a large number of preparations that the above description of the development of the embryonal cavity is the correct one. It is probable that Hegelmaier was misled by a section somewhat out of the median plane, from a seed in the stage represented by Fig. 7, which would show two cavities separated by the tissue which surrounds the narrow neck. A comparison with Fig. 6 shows that there is one continuous cavity before the widening of the apical part of the sac, and such a section as that supposed is possible only after this widening.

The adult seed of *C. indica* reaches a length of 9 to 10 mm., but, as may be seen from the above table, the preponderating growth of the chalazal part ceases when about half the final length of the seed has been reached. After this time the proportion between the two parts does not vary greatly. As the seed grows, the original ovular integuments form smaller and smaller portions of the whole, and in the ripe seed they occupy only a small region at the micropylar end. Here they furnish the tissue from which the seed-coat is differentiated, while the remainder of the testa is formed from the external chalazal tissue. Between the seed-coat and the embryo-sac is a mass of tissue which, in the ripe seed, contains starch, and has been called perisperm. It is, perhaps, not necessary to give it any other name, though the usual definition of perisperm as a storage-tissue derived from the nucellus does not include it. This development of the bulk of a seed from the chalazal portion of the ovule is a rare and remarkable phenomenon, although Kayser ('93) has described somewhat analogous features in the development of the seed of *Tropaeolum*. It is difficult to conceive what influences can have brought about so peculiar a modification in a few isolated cases.

Having now obtained a view of the general course of development in *Canna*, we may proceed to examine its histological details.

The seed-coat or testa is developed from both integuments of the ovule and from the layers of the chalaza into which the integuments pass at their bases. The inner integument is, at the time of fertilization, of a nearly uniform thickness. The micropylar portion lying above the apex of the embryo-sac becomes soon thereafter so overgrown and pressed together by the outer integument that the micropyle is tightly closed (*m.*, Fig. 6); and with continued growth it becomes less and less easily recognizable (*m.*, Fig. 10) until finally it can be identified only by comparison with earlier stages. When the young seed has reached a length of about 1·25 mm., there begins an unequal growth in the region of the base of the nucellus which results in an upward turning of the lower end of the inner integument (Fig. 6), heretofore directed downward. This bend becomes more and more pronounced, though but a small part of the integument is involved. When the seed is about 3 mm. long, there begins in the chalazal tissue the differentiation of a double cell-layer abutting directly upon the end of the inner integument and bending downward around the seed at a uniform distance from the surface and just within the vascular bundle (*ch. i. i.*, Figs. 8, 16). Thus what may be called the outer and inner coats of the chalaza are marked off; and, as they are in unbroken connexion with the original integuments, the subsequent differentiation of the whole testa proceeds uniformly. The discrimination of that part of the adult testa which is formed from the true integuments from that part developed from the chalazal coats is possible only by the study of its development. The inner integument forms in the ripe seed the internal tissue of the testa, consisting of two rows of large, empty, thin-walled cells, those of the inner row with colourless walls, while those of the outer row have amber-coloured membranes (*ch. i. i.*, Fig. 19). The outer integument of the chalaza is twelve or fifteen cells in thickness. The outer layer is composed, from the first, of cells slightly elongated at right angles to the surface (*ext.*, Fig. 15 a). As the seed grows these cells continue to elongate, forming a palisade-layer; and when the adult size

is nearly reached, their radial walls begin to thicken. This thickening is greatest at first at their outer ends (Fig. 18 a), but finally the whole cell-lumen becomes obliterated except a small cavity at the base filled with granular remains of the cell-contents, and a smaller space at the outer end. A faint line connecting these remnants of the cell-cavity marks the place where the thickening walls have met (Fig. 19 a). The appearance of a well-defined 'light line' at about one-third of the length of these cells from their outer ends (*l. l.*, Fig. 19 a) marks them as the true 'Malpighian cells,' so characteristic of the coats of many seeds. Overhage ('87) states that the walls in the region of this line are lignified, as Mattirolo has shown to be true for many other seeds. When these walls begin to thicken, those of the four or five cell-layers just below commence the same process (*scl.*, Fig. 18). This leads to the development of the second histological element of the testa, the sclerotic layer (*scl.*, Fig. 19). The remaining tissue of the outer coat remains thin-walled (*nut.*, Fig. 18), and during the ripening of the seed is gradually compressed, until there remains only a thin band in which the original cellular structure is hardly recognizable (*nut.*, Fig. 19). It seems probable that these cells furnish material for the characteristic development of the others, and that the name 'nutrient layer,' applied to them by Holfert ('90), is appropriate. The basal part of the palisade-layer, the walls of the sclereids in a slight degree, and especially the compressed nutrient layer, acquire during the ripening an increasingly dark brown colour, so that the ripe seed appears nearly or quite black to the naked eye.

While the foregoing description applies to the body of the seed, there are some respects in which the structure of the coat at the micropylar end requires special discussion. When the seed is about half grown, the downward bend of the inner integument about the apex of the embryo-sac becomes longer and thinner, forming a sharp projection of the seed-coat into the perisperm, which may be termed the micropylar collar (*m. c.*, Fig. 12). This peculiar collar, which we shall find to be very characteristic of the Scitamineae, differs slightly in

its histological details from the rest of testa. We have seen that the end of the original inner integument is bent upward before the differentiation of the chalazal integuments. In accordance with this we find that the inner face and the extreme lower portion of the outer face of the collar are formed from the true integument (*i. i.*, Fig. 14), while the rest of the outer face is formed by chalazal integument. Before the ripening of the seed the integument of the inner face is compressed to a mere line, and the double layer of cells which is elsewhere seen is here unrecognizable. Thus the true inner integument of the ovule becomes practically obliterated, with the exception of the bit of its originally lower end which is upturned at the lower edge of the collar. Beneath the compressed inner integument occurs a dense small-celled layer not elsewhere found (*x*, Fig. 14). The nutrient layer is here much less compressed than in the rest of the testa, but is readily disintegrated when the seed is ripe. The sclereid layer occurs in the collar as elsewhere, and, with the dense inner layer, forms its substantial portion. In but one region can any discontinuity in the tissues of the testa be observed. From the tip of the embryo-sac a break through all its layers can be traced to the surface, where it is recognizable as the tiny crack whose morphology has been already pointed out, and which has been called the germinal slit (*g. s.*, Fig. 14). As has been shown, the funiculus is attached at this point (Fig. 11); and if a seed be carefully detached from the placenta, its whole micropylar end is found to be covered by a structure which greatly resembles some of the arillar developments of other Scitamineae (*f*, Fig. 12). Examination shows, however, that it is a fimbriate outgrowth from the free portion of the funiculus, quite external to the seed.

When germination begins with the swelling of the embryo in the seed, the pressure must soon cause a rupture at the weakest point. This is evidently the point which, for this very reason, we have called the germinal slit. As this crack is stretched open, the testa breaks on either side, and this break follows a quite circular course, so that a small disk-like

lid is punched out at the micropylar end, permitting the escape of the embryo. The circular form of the lid appears to be determined by the firm ring-like collar surrounding the apex of the embryo-sac. The line of least resistance is evidently at its inner face, so that it comes to form a close collar about the neck connecting the growing part of the embryo without the seed, with the absorbing organ that remains within. Except at the germinal slit, I have been unable to recognize any sign of preparation for the cutting out of the lid. Yet it seems remarkable that, without some differentiation, it should always show the clean-cut inward bevel it possesses. It may be observed that Hegelmaier's ('74) statement that the whole of the testa formed from the original integuments of the ovule constitutes the lid is here shown to be inexact. This lid was early observed by Mirbel ('10), and more recently by Gris ('64), Hegelmaier ('74), and Klebs ('85). Its existence is denied by Tschirch ('90), who states that there is merely a sickle-shaped break in the sclereids of the coat, formed by the separation of the lower ends of the sclereids. The name 'sclereids' is here evidently applied to the cells of the palisade-layer. This statement concerning the absence of a lid in *Canna* is correct only in so far as it refers to such a preformed one as is found in many other Scitamineae.

With the exception of a kite-shaped region at the micropylar end, which includes the germinal slit (Fig. 13), the surface of the testa is thickly marked with tiny dots, as seen by the naked eye. Moderate magnification shows these to be true stomata, as was first pointed out by Schleiden and Vogel ('42). In the early stages of the development of the seed, their guard-cells are living, and they are evidently capable of performing the functions of stomata (Figs. 20 a, 20 b, Pl. II). As the outer layer becomes transformed into a palisade-tissue, the guard-cells also lose their cell-contents and become rigidly fixed in the wide-open position. They remain but slightly sunken below the surface, but their air-cavities become greatly lengthened by the growth of the palisade-cells (Fig. 21). This occurrence of stomata in the seed-coat

is a rare phenomenon, and suggests interesting physiological inquiries as to its significance. *A priori* it is not easy to see what special need of this particular seed should have led to the development or preservation of these structures in so unusual a place. So far as one can judge from their structure and that of surrounding cells, they can hardly remain functional much later than the time when the seed reaches about half of its adult length. Schleiden and Vogel ('42), Haberlandt ('84), and Overhage ('87) have suggested that these open pores furnish the only means for the penetration of water into the seed at the beginning of germination. Against this idea it may be urged that they are completely closed below by the sclerotic layer of the testa, beneath which is the dense nutrient layer: and further, the equally dense coats of other seeds have no stomata. It is much more probable that water can penetrate into the seed of *Canna* through the germinal slit.

The chalazal perisperm lying between the testa and the embryo-sac becomes, like the perisperm of other seeds, a storage-tissue. The perisperm-cells become gradually elongated in a radial direction, beginning with those which border on the embryonal cavity; and soon starch is deposited in characteristic grains in them, until all are filled, those next to the testa last. Concomitantly with the deposition of starch in the cells, their walls become slightly thickened and pitted. So completely are the cells filled with starch that the impressions of the grains upon the walls are very evident after their emptying during germination (*psp.*, Fig. 24).

We have now to notice the two tissues developing within the embryo-sac. The fertilized egg-cell develops directly into the embryo without the formation of a suspensor, Hofmeister's statements to the contrary notwithstanding. Details of its development were not followed out, since they have been sufficiently described by Hegelmaier ('74). The adult embryo is remarkable for the high degree of differentiation which it reaches, especially in its plumule and in the number of accessory root-rudiments. When germination begins, the elongation of the neck of the absorbing organ pushes out the

growing part of the embryo, and some of the accessory roots quickly outstrip the primary one. A discussion of the various interesting features of germination in *Canna* is reserved for a future occasion.

The endosperm. The nucleus, formed by the fusion of the polar nuclei, has been said by Hofmeister ('49, '61) to give rise to no endosperm; the statement is repeated by Tschirch ('90), and by various text-books. But a short time after fertilization a definite and readily separable protoplasmic layer with numerous nuclei may be found in the micropylar part of the embryo-sac, precisely as it occurs in plants with abundant endosperm (*csp.*, Fig. 9). As development proceeds, this layer may be followed downward until, when the seed reaches its full size, the entire cavity may be found to be lined by it. There now appear cell-walls within the layer, but not so regularly as in many plants, since several nuclei are commonly included in a single block of cytoplasm (Fig. 22). Ultimately each cell becomes uninucleate, apparently by a process of nuclear fusion similar to that observed by Strasburger ('80) in *Corydalis* (Fig. 23). In the ripe seed this layer assumes all the characters of the so-called 'aleurone-layer,' now known in the seeds of so many plants, and lies between embryo and perisperm, in contact with both. Traces of this layer seem to have been recognized by Hegelmaier ('74) in early stages of the seed; but he failed to discover its presence in the ripe seed. Overhage ('87), on the other hand, correctly describes its occurrence in the ripe seed and suggests that it may be a remnant of endosperm, but without presenting any evidence of the correctness of his suggestion. This appears to be the first recorded case of the normal reduction of the endosperm to a single persistent aleurone-layer, and points to this tissue as one of considerable importance. Guignard has lately shown ('93) the very wide distribution of such a layer in so-called exalbuminous seeds; but in these it appears to be always a remnant of a more voluminous endosperm. In other cases in which the development of endosperm does not exceed the formation of a single

parietal layer, this usually becomes disintegrated without the production of cell-walls, as in the Vicieae, *Limnanthemum*, &c. The persistence of the single layer and its acquirement of the character of an aleurone-layer strengthens the probability indicated by Guignard's results, that it has a special physiological significance. This seems especially probable in the light of the recent researches of Grüss ('95), confirmatory of Haberland's earlier view ('90) of the aleurone-layer as a diastase-producing tissue.

In comparing the seed-development of *Canna* with that of other Scitamineae, we may first examine those nearest to it in floral structure, the

Marantaceae.

If any suggestions as to the origin of the peculiar developmental history of the seed of *Canna* are to be found, one would expect them from Marantaceae. Material of even the chief stages in the development of the seeds of these plants is very difficult to procure; and, in spite of many efforts, I have not succeeded in obtaining a complete series of any member of the family. Of most forms seen I have been able to examine only the ovules at about the time of flowering, or the ripe seeds. The discussion of the family must, therefore, be very incomplete.

The ovules are very uniform in structure and position throughout the family. Their position has already been described. Each ovule shows from an early stage (Fig. 25) a greater development of the outer than of the inner side of the nucellus and of the corresponding parts of the integuments; so that the nucellus becomes bent around against the funiculus, as in an anatropous ovule, while its morphological base, at the chalaza, is not opposite the micropyle, but at one side (*ch.*, Fig. 26). Thus the ovule is neither typically anatropous nor campylotropous. Its attachment is by a short and fleshy portion into which the vascular bundle passes from below, and which must be regarded as a much thickened funiculus. The ovules of members of the genera *Maranta*,

Calathea (Fig. 32), *Myrosma*, and *Thalia* (Fig. 26) show essentially the same structure, and this probably holds true for the other genera of the family. A section of the embryo-sac from an ovule of *Thalia dealbata* ready for fertilization shows the usual arrangement within the sac in a very clear manner (Fig. 27).

The subsequent development of the ovule into the seed is such as to give the seed a truly campylotropous character, with a curved embryo-sac and embryo, in all but one of the forms examined. Certain features of this development have been described by Schleiden and Vogel ('42), by Gris ('59, '60, '60[a]), and by Eichler ('84); and the structure of the adult seeds has been discussed by Nees von Esenbeck ('31). But no connected account of the development has been given, nor can the deficiency be here supplied. In those genera whose flowers produce but a single ovule each (*Maranta*, *Thalia*, &c.), the cavities of the sterile loculi become reduced to almost or quite imperceptible slits. The most satisfactory material at my disposal was that of *Thalia dealbata*, Fraser, to which the following account refers.

The inner integument of the ovule, which, at an early stage, is but slightly unequal on opposite sides of the nucellus, and regularly curved (Fig. 25), soon begins to show a sharp inbending at a point about opposite the base of the embryo-sac. This is often more marked on the short side of the integument (Figs. 26, 32). The inbending continues as growth proceeds, with the result that the proportional distance from micropyle to chalaza is steadily reduced (Fig. 29). Meantime that part of the nucellus beyond the chalaza from the micropyle, with its integuments, is growing around the chalaza and curving downward (Fig. 29). Thus finally the chalaza is brought down close to the micropyle, as in seeds derived from truly campylotropous ovules (Fig. 30). During this process the embryo-sac elongates into the distal part of the nucellus, curving with its growth, until when fully developed the base of it reaches nearly to the chalaza, and the whole cavity assumes the form of a horse-shoe. The

cells along the future course of the cavity begin quite early to show signs of degeneration, and a dark band extending from the base of the embryo-sac first marks the path of its extension (Fig. 29). Early in the development of the seed, two cylindrical differentiations of slightly denser-looking tissue may be seen in the chalazal region of the nucellus, curving slightly and lying parallel with each other on either side of the plane of symmetry of the seed, but evidently originating in the nucellar tissue itself (*p. c.*, Fig. 29). They begin abruptly in the region of the base of the inner integument. These two cylinders then follow in their development the extension of the embryo-sac, so that there are formed two parallel tissue-masses curving with this cavity, but just within its curve, and lying on either side of its median plane (*p. c.*, Fig. 30). This tissue finally consists of loose, brown and dead cells, through which run branches of the vascular bundle which earlier reaches only to the base of the nucellus (*v. b.*, Fig. 29). In the adult seed these two 'perisperm-canals' appear as branches from a simple projection into the perisperm from the chalazal region. This seems to be a mass of chalazal tissue which has been included by the growth of the nucellus, and strictly homologous with the similar included tissue-masses shown by Meunier ('90) to occur in the seeds of various Centrospermae. These latter seeds are developed by growth similar to that observed in the Marantaceae from similar ana-campylotropous ovules. Unfortunately, just the stages lacking in my material are the important ones between Figs. 29 and 30, during which the differentiation of the canals takes place. This remarkable development of the perisperm-canals in *Thalia* is probably their most extremely developed condition in the Marantaceae; but they form a very characteristic feature of the family, and perhaps no strictly homologous structures are yet known in other plants. In most genera of the family there is but a single canal, extending inward between the arms of the embryo-sac and in the same plane. The first speculations as to its morphological significance appear to have been those of Robert Brown ('10), who suggested that

the canals of *Thalia* represent sterile embryo-sacs. This idea was shown to be mistaken by Gris ('59, '60, '60[a]), who considered them to be ingrowths of the chalazal tissue into the nucellus. Eichler ('84) added only a few details concerning the adult structure of certain seeds, and regarded the canals as chalazal ingrowths. Tschirch ('90) refers to them merely as ingrowths of the seed-coat, which they certainly are not. In the absence of full details of their development, we are not in a position to decide finally the question of their morphology. Yet the fact that they extend inward for a considerable distance from the chalaza, on their first appearance, and that they seem to arise as gradual differentiations in the nucellar tissue, directly opposes the view of Gris and Eichler, except in so far as the common basal portion of the canals in *Thalia*, which is probably present in other forms also, is due to the enclosure of chalazal tissue by the growth of the nucellus, as in the Centrospermae. The conception of the chalaza as an actively penetrating *organ*, like the embryo-sac, rather than as a mere *region* of the seed will, I think, hardly bear criticism. It seems, then, most probable that the perisperm-canals are in part specialized portions of the nucellus, and in part chalazal tissue included by the growth of the latter. Of what use they are to the seed future studies must attempt to explain.

In a single Marantaceous seed, whose development I have not been able to study, the embryo is not hooked, but nearly straight, and lying parallel with it is the perisperm-canal (Fig. 32 A). Whether we have here a seed developed without the campylotropic growth usual in this family, it is impossible to say. If not, then, for some reason, the extension of the embryo-sac into the later-formed portion of the nucellus has been here suppressed. If so, we have evidence that this growth is not necessary to the formation of a perisperm-canal. The seed here mentioned, whose development promises to prove of considerable interest, is that of *Phrynium capitatum*, Willd.

Returning to our study of *Thalia*, we may note some details

of the development of the tissues of the seed. The single-seeded fruit of this genus is indehiscent, and the seed is apparently set free by the decay of the rather thin pericarp. The outer integument of the ovule is about six cell-layers in thickness (*o. i.*, Fig. 28), of which the outer and inner layers begin early to take a distinctly cuboidal form. The cells of the intermediate layers increase irregularly in number, and constitute a nutrient layer which shows compression and obliteration of its cell-cavities as the seed ripens (*nut.*, Fig. 31). In the ripe seed the cells of both outer and inner layers are large and thin-walled, while the latter show traces of compression in the wavy folding of their radial walls (*ext.*, *int.*, Fig. 31). The inner integument is here pressed into a film in which no cellular structure is recognizable (*i. i.*, Fig. 31). The infolding of the inner integument before mentioned (*m. c.*, Fig. 29) produces the same result as in *Canna*—the formation of an enclosing testa-collar about the apex of the embryo-sac (*m. c.*, Fig. 30). In many Marantaceae a distinct germinal lid is said to be developed over the end of the cavity. In *Thalia* I have been able to recognize no such differentiation, possibly from lack of sufficiently old seeds. *Thalia dealbata* possesses no trace of an aril; but in most members of this family, which Eichler ('84) has remarked to be those with dehiscent fruit, the thickened funiculus, with the tips of the integuments, forms a fleshy arillar appendage to the seed, which has been shown by Fritz Müller ('83) to play an important part in the dehiscence of the fruit and the expulsion of the seeds. Eichler remarks ('84) that the origin of the aril in this family is peculiar; but just those parts are here concerned which we shall find contributing to the aril in other Scitamineous families. The most unusual feature here is the extreme thickening of the funiculus.

The large amount of nucellar tissue that is neither destroyed by the embryo-sac nor used for the perisperm-canal becomes filled with starch to form a perisperm. The embryo ultimately attains the form and size of the embryo-sac, usually tapering towards its cotyledonary end. In young seeds of

Thalia I have observed what appears to be a young endosperm-layer, but have not been able to recognize any trace of such tissue in the older seed. The material, however, is insufficient to justify a positive statement as to the formation of either temporary or permanent endosperm in these seeds.

Although most recent writers separate the Cannaceae from the Marantaceae, they are still united by some into a single group. Apart from the marked differences in ovary and fruit between *Canna* and the other genera, the striking uniformity and peculiar form of the ovules in all of the latter so far as known, the development of the body of the seed chiefly from the nucellus of the ovule, and the formation of perisperm-canals, as compared with the striking divergence of *Canna* in all these respects, seem to afford ample ground for the separation of the latter genus as the type of a distinct family. Certain peculiarities common to these two families will be found to be shared by other Scitamineae, now to be described.

ZINGIBERACEAE.

Passing now to this largest, both in genera and in species, of the families of Scitamineae, we find, nevertheless, that the structure and development of its seeds have received still less attention than in any other of these families. Except in *Globba* and the related genera, which have a unilocular ovary with three parietal placentas, the gynaeceum shows the structure most common in the Scitamineae. Each ovary normally forms a dehiscent capsule with numerous seeds, which are commonly so closely packed as to be irregularly angular from mutual pressure. The most complete material studied was that of an undetermined species of *Costus*, which may be taken as the basis for the discussion of the family. Less complete material of *C. speciosus*, Sm., agreed in all essentials with the former, so far as comparison was possible.

The ovule shows, at a very early stage, before the integuments have begun to appear, a sub-epidermal archesporial cell (*a*, Fig. 41, Pl. III), distinguished by its greater size. The tapetal

cell is soon cut off (*tap.*, Fig. 42), and then divides by anticlinals into a series of cells similar to those of the body of the nucellus (*tap.*, Fig. 43). The mother-cell of the embryo-sac enlarges as the ovule grows, but does not divide further, and thus becomes itself the definitive embryo-sac (*e. s.*, Figs. 43, 44).

The development of the seed from the ovule follows the usual course in its general features, but certain details will repay examination. The integuments consist of about six and two cell-layers, respectively. The inner coat becomes finally reduced to a thin remnant (*i. i.*, Fig. 49). The cells of the external layer of the outer one become elongated parallel to the surface of the ovule, while those of the internal layer lengthen radially (*ext.*, *int.*, Fig. 47), and become the chief protective layer in the ripe testa. When fully developed they are empty and have somewhat thickened inner and radial walls, while their outer walls remain thin. Peculiar modifications of the lateral walls give to this row of cells a very characteristic appearance, best understood from Fig. 49 a. The cells included between the outer and inner layers constitute a nutrient layer and become greatly compressed during ripening (*nut.*, Fig. 49 a). Just after fertilization, the free end of the outer integument and the free portion of the funiculus into which it passes at one side of the micropyle begin to swell (*ar.*, Fig. 45), and gradually close the micropylar opening, compressing, and finally obliterating, the end of the inner integument. The mass formed by the coalescence of the thickenings grows rapidly, becoming very large and fleshy, and finally forms the aril which caps the micropylar end of each seed (*ar.*, Figs. 46, 48). From the mode of its formation it is evident that it must include the funicular bundle and the remnant of the micropyle; and these may be recognized in all stages (*m.*, *v. b.*, Figs. 46, 48). This micropylar aril is of the type to which Planchon ('45) has given the name *arillode*.

At an early stage the inner integument has a regular ovoid curve (*i. i.*, Fig. 44); but about the time of fertilization the broadening of the nucellus gives it a prominent shoulder at the outer angle (Fig. 45). Just within this shoulder the

integument now begins to bend downward into the body of the nucellus, thus giving rise to the micropylar collar which surrounds the apex of the embryo-sac, as in *Canna* and *Thalia*. The outer face of this collar shows the characteristic inner layer of the seed-coat, but this layer disappears at the edge of the fold which forms the collar, so that its inner face remains thin. Meanwhile there is formed just over the embryo-sac, from tissue of the outer integument, and around the micropylar remnants as a centre, a circular lid whose edge abuts on the upper margin of the collar (*g. l.*, Fig. 48). In the adult seed this lid is a firm, resistent tissue, and rests upon the edge of the testa so as completely to close its opening, without being in organic continuity with it. Thus the embryo is well protected, yet is able to push out the lid easily, when germination begins, since no dense tissue requires to be ruptured.

At fertilization the chalazal end of the inner integument lies about transverse to the axis of the nucellus (Fig. 45). Soon it begins to turn upward, and at length a small part of the end of the integument stands almost at a right angle with the adjoining portion (Fig. 46). The tissue enclosed within this upturned end becomes specially differentiated, while the integument itself becomes compressed, as elsewhere. A continuation of the inner layer of the outer integument is developed as far upward as the inner integument extends (*int.*, Figs. 48, 49). The cells of that part of the outer layer lying over this region become thicker and much shorter, so that they form here a palisade-like layer much denser than on other parts of the surface. This peculiar discoid patch at the chalazal end of the seed is plainly evident to the naked eye. The bounding layers of the region are lined by a double layer of cells with thickened walls, and within these, and filling most of the region, is a compact tissue of small thin-walled cells (*ch. m.*, Fig. 49). The possible significance of this peculiar development of chalazal tissue will be discussed later.

Within the seed-coat the cells of the perisperm are considerably elongated (*psp.*, Fig. 49 a); but toward the centre

they become much less so. As in other plants, they are closely packed with starch in the mature seed. Even before fertilization the cells of the micropylar part of the nucellar epidermis begin to elongate radially, so that a thick pad is formed at this point, through which the pollen-tube must penetrate (*m.p.*, Fig. 45). Such a pad may be seen in the Marantaceae, but less markedly developed than here. It continues to thicken for some time after fertilization (*m.p.*, Fig. 46), but finally becomes much compressed by the growth of the embryo, though still recognizable in the ripe seed (*m.p.*, Fig. 48). After fertilization the embryo-sac enlarges at first far more rapidly at the base than at the apex (Fig. 46), but finally broadens above also to the full width determined for it by the micropylar collar (Fig. 48). Within this cavity the almost cylindrical embryo is formed, showing evident rudiments of two or more leaves of the plumule and of accessory roots (*emb.*, Fig. 48). As in *Canna*, there is no suspensor. Unlike that of the previously described families, the endosperm of *Costus* and of other Zingiberaceae, perhaps of all, reaches a considerable thickness, although it never wholly fills the cavity. In the ripe seed of *Costus* it forms a single cell-layer over the micropylar end, and a layer several cells thick upon the wall of that part of the sac below the edge of the micropylar collar, more or less completely filling the space between the embryo and the wall of the cavity. As Tschirch ('90) has remarked, the cells of this permanent endosperm contain no starch, but aleurone. So that we have here, instead of the single aleurone-layer of *Canna*, an aleurone-mass. But this fact need not modify our view of its rôle in the economy of the seed.

In comparing members of other available genera of this family with *Costus*, several interesting points of difference are worthy of note. In the differentiation of the chief layer of the testa from the inner layer of the outer integument, in the presence of the micropylar collar and germinal lid, and in the development of embryo and endosperm, all the forms examined agree closely. In *Donacodes elongata*, Teijsm. et

Binn. (=*Amomum*), *Elettaria* sp., and *Alpinia mutica*, Roxb., the characteristic inner layer of the outer integument continues over the apex of the embryonal cavity, thus forming both faces of the collar and the germinal lid (Fig. 37). At the point of junction of lid and collar there is a break in the continuity of the layer, which makes it easy for the embryo to push out the lid in germinating. It will be noticed that, in the formation of collar and lid, all these genera differ from *Costus*. The form of aril above described has been met with only in *Costus*. On ripe seeds of *Amomum*, *Elettaria*, and *Alpinia* one finds no marked micropylar excrescence, but a soft, loose, veil-like covering over the whole seed (*ar.* Figs. 37, 39), which is attached to the underlying tissues only at that end. An examination of the ovules shows the origin of this enveloping structure. From the micropylar end of the outer integument arises a downward outgrowth, and from the inside of the funiculus just above the micropyle arises another outgrowth which extends across the micropyle and then downward over the first-named one. From the back side of the funiculus arise two corresponding outgrowths that extend downward and form a double covering for that side of the ovule (*ar.*, Fig. 34). That the adult aril is really double may be seen on careful examination near the point of origin (*ar.*, Fig. 39). The ovules of *Alpinia alata*, A. Dietr., and *Globba bulbifera*, Roxb., show similar outgrowths, and probably their seeds have similar enveloping arils. The use of this form of aril to the seed is not easy to suggest. The aril of *Costus* much resembles those of the Marantaceae, and may well play a part in the rupture of the capsule and the escape of the seeds. And it is possible that this soft, yielding envelope outside of the firm testa may, when fresh, have the power of swelling and causing the bursting of the fruit.

The seeds of *Amomum* and *Elettaria* show no sign of the dense chalazal mass found in those of *Costus*. But the ovules of *Globba* show the beginning of a similar development; while in the seeds of *Alpinia mutica* it is carried much farther than in *Costus*. On the nearly ripe seed of this species there may

be seen a slight groove extending from the micropylar end on either side downward around the chalazal end. A section at right angles to the plane of this groove (Fig. 39) shows an ingrowth from the chalazal region, reaching to about the middle of the seed. At its apex this ingrowth broadens slightly. If the seed be cut in the plane of the groove first mentioned (Fig. 40), it is found that the ingrowth before seen is a section of a dense testa-like diaphragm, extending across the chalazal half of the seed, and that the groove marks the line of its union with the testa: above the upper limit of the diaphragm, it is continued to the micropylar end by a slight projection of the testa into the perisperm. The diaphragm is sharply bounded against the perisperm by a cell-layer continuous with, and similar to, that derived from the inner layer of the outer ovular integument. This layer stops abruptly just below the top of the thickened upper edge of the diaphragm, and it may be observed that the thickening of this edge is due to a sudden outward bending of this bounding layer (Fig. 39), strikingly like the upward bend which limits the chalazal mass of *Costus* (Fig. 46). It is to be regretted that my material contains no intermediate stages between the practically adult one just described and young ovules (Fig. 38). These latter show the usual structure, and give no hint of the origin of the diaphragm. But, from the homology of *Costus*, it may be suspected that its formation is begun by the ingrowth of the lower end of the inner integument, with perhaps an infolding at the sides to meet the upward growth. The development of this interesting seed would probably prove well worth following on suitable material. The intrusion of this dense sheet of tissue into the region usually occupied by the embryo-sac causes the latter to spread out over the top and then to extend downward on either side of the diaphragm (*e. s.*, Fig. 39). The embryo-sac becomes largely filled with endosperm, and the lower part remains so, as the embryo does not completely fill it. Tschirch ('90) has remarked that the embryo of *Alpinia nutans* is two-lobed in a sickle-

shaped endosperm, which probably refers to a condition similar to that here described. It is probable that in the chalazal mass of *Costus* and the more striking diaphragm of *Alpinia* we have homologous structures of which a thorough study of Zingiberaceous seeds would bring to light other forms and grades of development. The thickened edge of the ingrowth in *Alpinia* seems to correspond to the rounded mass in *Costus*. In the former genus the chalazal mass has penetrated far into the seed, probably as the result of a downward extension of nucellar tissue on both sides of it. The use of these structures to the seed cannot be shown without studies on their germination; but the possibility suggests itself that they may serve to afford firm points of support from which the embryo may exert its full pushing force upon the germinal lid at the beginning of germination. We have seen that the original differentiation in the Marantaceae extends inward from the base of the nucellus, with no accompaniment of the inner integument. In *Costus*, on the contrary, and apparently in *Alpinia*, this integument does limit the ingrowth. Whether all the tissue that forms the diaphragm in *Alpinia* is chalazal cannot be determined without a study of the development of the seed. But it does not seem likely that these structures in the Zingiberaceae can be strictly homologized with the perisperm-canals of the Marantaceae, though they may correspond to the chalazal portions of the latter. The suggestion above made as to the possible use of the chalazal mass in the former family is not so obviously applicable to the true perisperm-canals.

We come now to the last of the Scitamineous families to be discussed, the

MUSACEAE.

In this family of four genera and comparatively few species, the members of the genus *Heliconia* present the most interesting and aberrant features. It is well known that several species of *Musa* develop seeds rarely or not at all. Of those which do so, *M. Ensete*, Gmel., has been studied by Wittmack ('68),

who describes only the structure of the ripe seed. I have been able to examine ovules and young seeds of about adult size of a species sent from Jamaica under the name *M. rosea*, but could not follow the development of the former into the latter. The ovule (Fig. 50) presents nothing noteworthy, except a dense felt of long simple trichomes arising from the sides of the funiculus. No trace of these remains in the seed. As compared with the ovule, the seed (Fig. 50 a) shows great lateral extension, so that the embryonal cavity is much shorter than broad. The trace of the micropylar opening can still be recognized (*m.*, Fig. 50 a), and, as might be expected from the indehiscent fruit, no aril is developed. The outer cell-layer of the testa forms a deep palisade-layer, and its inner layer consists of large cuboidal cells. Between these two the cells become much elongated parallel to the surface, and the walls of a few of the outer layers become conspicuously thickened and pitted. Whether the remaining cells constitute a nutritive layer and finally become compressed could not be determined. At the chalazal end the inner integument is bent upward, and my material points to the differentiation of a chalazal mass similar to that of *Costus*. Wittmack's ('68) figure also indicates the presence of such a structure in *M. Ensete*. The micropylar collar so characteristic of most Scitamineae is here present also; and its outer end is closed by a germinal lid differentiated from the inner layer of the outer integument, as in *Amomum* and other genera.

The epidermis of the nucellus thickens into a conspicuous micropylar pad (*m. p.*, Fig. 50 a). The inner nucellar tissue is largely obliterated by the growth of the embryo-sac, but in the oldest seeds examined there is still a broad band of perisperm present, though the deposit of starch in its cells has not yet begun. Within the cavity neither endosperm nor embryo could be found, but without more complete material it is impossible to say that this species forms only abortive seeds. The form of the cavity would determine for the embryo, if developed, the fungiform shape observed in other species of *Musa*.

Strelitzia Reginae, Ait., shows some points of interest in the structure of its seeds, which appear not to have been studied since the time of Gaertner (1791). The ovule (Fig. 51) is not peculiar. The inner integument becomes reduced to a thin layer with the exception of its thickened micropylar end, which is recognizable until the seed reaches its adult size (*i. i.*, Fig. 53). The outer integument attains a considerable thickness and consists of many cell-layers (Fig. 54). Of these the cells of the inner layer are finally cuboidal and thin-walled (*int.*, Fig. 56). Those of the outer layer take a similar form, but remain smaller and thicken their walls until but a very small lumen remains (*ext.*, Fig. 56). All the intermediate tissue constitutes a nutrient layer which becomes reduced in the ripe seed to a thin, dense mass (*nut.*, Fig. 56), the compression first showing itself next to the outer layer (*nut.*, Fig. 54). The chalazal end shows no special differentiation, and no trace of the micropylar collar, so usual in this group, is found. At the micropylar end there appear, even before fertilization, papillar outgrowths from the free end of the outer integument and from the part of the funiculus lying opposite (*ar.*, Fig. 52). These elongate into the multicellular fibres which in the ripe seed form two dense woolly tufts, of a deep orange colour, at the micropylar end (*ar.*, Fig. 55). The structure of these arillar fibres has lately been described by Pfeiffer ('91). They arise from the sides of a caruncle-like outgrowth formed by the coalescence of the ovular regions from which the outgrowths spring. The body of this caruncle consists of undifferentiated tissue which is directly continuous with the nutrient layer of the testa, but which, unlike the latter, preserves its cellular structure. Up to the point where the arillar threads begin, the surface is protected by an outer layer, like that of the testa. This aril is evidently quite homologous with those of other Scitamineae, the differences being merely in detail. I can add nothing to the speculations of Pfeiffer ('91) and others concerning the use of these brilliant woolly tufts.

The embryo-sac increases in size during the development

of the seed to such an extent that only a thin layer of nucellar tissue is left within the seed-coat, and this soon loses its cellular structure by compression (*psp.*, Fig. 54). A massive endosperm is formed in the cavity, but never wholly fills it. In the centre of the cavity is formed the almost cylindrical embryo (*e. s.*, Fig. 55), and the space between it and the remnant of perisperm is occupied by a permanent endosperm containing starch except in its outer cell-layer, which forms an aleurone-layer. In the ripe seed the perisperm forms a mere film against the testa (*psp.*, Fig. 56). In this species, then, we have a Scitamineous plant without functional perisperm.

I have examined neither of the species of *Ravenala.*

The remaining genus, *Heliconia*, has furnished the most complete material studied from this family. The work has been done chiefly on *H. psittacorum*, L. fil., though *H. Bihai*, L., shows the same features so far as comparison has been possible.

Except for a few figures given by Richard ('31) of his *H. cannoidea*, which well show the general appearance of fruit and seed, and some similar, though not better, figures by others, I have found no references in the literature to the seeds of this genus. The ovary becomes a three-seeded septicidal capsule.

In the earliest stage observed, the ovule shows both integuments in process of formation and an embryo-sac mother-cell which is separated from the nucellar epidermis by a tapetal row (Fig. 57, Pl. IV). A little later, this mother-cell has divided into three cells (Fig. 58), and still later into four (Fig. 59). Four is the usual number of cells in the row thus formed, but I have seen five in at least one case (Fig. 60). Soon the lower cell of the series begins to enlarge at the expense of the others (Fig. 61), and finally it becomes the embryo-sac by the absorption of all between it and the nucellar epidermis, which meanwhile and later thickens into a micropylar pad (*m. p.*, Figs. 63, 66). During this time the ovarian cavity has grown with the ovule, so that the latter has filled but a small

It is apparently by the breaking up of these empty cells that the exocarp is separated from the endocarp. The exocarp forms a firm and dry envelope about the seed, showing no pulpy consistency. At a pretty early stage the line of demarcation between exocarp and endocarp may be traced (Fig. 62) around the loculus, extending downward at the base to or just below the point of origin of the very short funiculus. Since the cleft which separates the funiculus from the ovarian wall extends farther downward on the side of the micropyle than on that of the raphe, the plane of the end of the endocarp is very oblique to the axis of the ovule. The abrupt termination of the endocarp in this region leaves at the micropylar end of the seed an unprotected circular area enclosed by its rounded end. This area is originally occupied by the soft parenchyma of the funiculus, which is pierced by the vascular bundle (*v. b.*, Fig. 64). After the seed has reached its full size and the endocarp is well formed, a differentiation begins in this tissue. The cells in an oblique band extending across the open mouth of the endocarp, just within its end (Fig. 68), begin rapidly to thicken their walls, and soon the mouth is filled by a thick plug of stone-cells thus formed (*scl. p.*, Fig. 69). When the seed is separated from its attachments the break occurs at the outer surface of this plug. It is probable that, in germination, it serves as a germinal lid, being pushed out by the growing embryo, while the end of the endocarp forms a firm collar about the neck of the absorbing organ.

The characteristic micropylar pad continues to enlarge (*m. p.*, Figs. 66, 68) until the seed reaches its adult size, and then suffers considerable reduction by compression (*m. p.*, Fig. 69). With the growth of the embryo-sac, the tissue of the nucellus is steadily reduced until only a narrow band next to the integument remains. This never becomes wholly obliterated, but remains as a distinct starch-bearing perisperm (*ps. p.*, Figs. 68, 69, 70). The walls of the embryo-sac become lined by a protoplasmic layer with free nuclei (*esp.*, Fig. 67), from which an endosperm-tissue develops in the

usual way. The massive endosperm finally fills the space between the perisperm and the cylindrical, slightly curved embryo (*esp.*, Fig. 69), and contains starch, like the perisperm. In this species, as in the other Scitamineae studied, the entire egg-cell contributes to the formation of the embryo, no suspensor being developed (*emb.*, Fig. 66).

General Results.

We may now compare the various Scitamineae above discussed for the purpose of determining in what respects they agree and what features of their seed-development can be considered characteristic of the group. We may also ask in how far closer affinities within the group are indicated by the facts brought out.

In the obliteration of the inner integument of the ovule during the development of the seed, all of the species studied, except *Canna*, agree with most other plants with bitegumentary ovules whose history in this respect is known.

Except in *Canna* and *Heliconia*, whose development is modified by aberrant features, the outer integument of the ovule gives rise to the chief part of the testa of the seed. As a rule, the outer and inner cell-layers are correlatively developed, the high development of one leading to the slight development of the other. Thus in *Canna* and *Musa* the outer layer forms a deep palisade-layer and the inner one is not specially developed. In the Zingiberaceae the inner layer is highly specialized, while the outer is a thin epidermis; and in the Marantaceae and *Strelitzia* each layer is moderately developed. In the presence of a nutrient layer in the testa the Scitamineae agree with great numbers of other plants of most various affinities. In both forms with a palisade-layer this is backed by a sclerotic layer, not elsewhere observed.

The micropylar collar and germinal lid appear to be closely related in their development, and to be very characteristic of the Scitamineous seed. The only observed instance of their absence not easily understood is that of *Strelitzia*.

Since the evident purpose of the lid is to facilitate germination, and that of the collar is to ensure the efficient connexion of the plantlet with the food-supply in the seed, and as the testa of *Strelitzia* is especially firm and shows no other special adaptation for these purposes, a study of the germination of this seed may prove of considerable interest.

While arillar structures are very common in those members of the group which have dehiscent fruits, they are commonly not at all developed in species with indehiscent ones. Whenever present, the aril arises from funiculus and integument in the micropylar region. It may form a mass at this end or an envelope about the seed.

Greater or less penetration of chalazal tissue into the nucellus appears to occur in *Musa*, in various Zingiberaceae, and probably in all Marantaceae. In *Costus* and *Musa* the chalazal differentiation has merely the form of a rounded mass, while in *Alpinia* there is formed a diaphragm penetrating to the middle of the seed; and in the Marantaceae the included chalazal tissue appears to be continued far into the seed by a differentiation (in *Thalia*, two) in the nucellar tissue. In how far the homologies here suggested are really valid must be determined by studies of the development of a variety of forms.

The formation of the true testa and of all the special structures associated with it in other forms is suppressed in *Heliconia* by the development of a stony endocarp. Here, apparently, the mouth of the endocarp replaces the micropylar collar, and the sclerotic plug replaces the germinal lid.

Even in *Canna* the pollen-tube must penetrate the nucellar epidermis to reach the embryo-sac, and in most of the species examined this portion of the epidermis is thickened into a firm micropylar pad, which reaches its greatest thickness after fertilization, and becomes most conspicuously developed in the Zingiberaceae and Musaceae.

In the plants examined, the starch-bearing tissue of the seed is entirely perisperm, except in the Musaceous genera. In *Heliconia* there is a narrow layer of functional perisperm

around the endosperm; while in *Strelitzia* the extreme reduction of this tissue to a useless remnant is realized.

The members of the Scitamineae present a progressive series of stages in the development of the endosperm. In the Musaceae this tissue is abundant and starch-bearing, though its outer cells may form an aleurone-layer (*Strelitzia*). In the Zingiberaceae the endosperm, though several cells in thickness in the lower part of the embryo-sac, contains only aleurone. In the Cannaceae this tissue is reduced to a single aleurone-layer lining the cavity; and in the Marantaceae it is probably not represented in the ripe seed.

The embryo-sac and embryo are practically straight in all of the Scitamineae, except most Marantaceae. In every species of the group examined, the entire egg-cell contributes directly to the formation of the embryo, without any development of a suspensor.

Coming now to questions of affinity, we find in the characteristic formation of the micropylar collar, in the persistence of the micropylar epidermis over the apex of the embryo-sac, in the very common development of a micropylar aril, and in the direct development of the embryo, the features of the seed which are to be regarded as most characteristic of the Scitamineae in general.

The uniformity of their ovules, the campylotropic development of their seeds, and the differentiation of their perisperm-canals, support the indications of their floral structure that the Marantaceae form a natural and closely related group of genera.

The Cannaceae show no near affinities in their seed-development with any other group of Scitamineae. In the development of the bulk of the seed from the chalazal portion of the ovule, in the coalescence of the micropyle and hilus to form the germinal slit, and in the presence of stomata in the testa, *Canna* stands quite alone. Therefore, while we fail to obtain light on the origin of these peculiarities or the phylogeny of the genus, we are quite justified in separating it as the type of a distinct family.

In the Zingiberaceae the striking differentiation of the inner layer of the testa forms the most marked common character. *Costus* differs from other genera examined in the form of its aril and in the histological character of its micropylar collar and lid. The form of the chalazal mass shows interesting variations; and studies of other genera are likely to prove very interesting, possibly indicating lines of descent within the family.

In respect to seed-development, the Musaceae are a heterogeneous group. Perhaps the nearest affinity with the Zingiberaceae is shown by *Musa*. *Strelitzia* lacks the most characteristic Scitamineous feature, the micropylar collar and lid, but is, in a measure, saved by its aril. In *Heliconia*, the supplanting of the testa by the endocarp precludes the supposition of a close affinity with the other genera.

List of Writings Referred to.

Brown, R., '10: Prodromus Florae Novae Hollandiae. London, 1810.

Eichler, A. W., '84: Beiträge zur Morphologie und Systematik der Marantaceen. Abhandl. d. k. Akad. d. Wiss. zu Berlin, 1883, I, p. 1, 7 pl., 1884.

Gaertner, J., 1788–'91: De fructibus et seminibus plantarum. T. I; Stuttgardiae, 1788: T. II, Tubingae, 1791.

Gris, A., '59: Note sur le fruit et la graine des *Maranta indica*, *Thalia dealbata*, et *Calathea villosa*. Bull. Soc. bot. de France, T. VI, p. 737, 1859.

——— '60: Origine et mode de formation des canaux périspermiques dans les Marantées. Bull. Soc. bot. de France, T. VII, pp. 237, 875, 1860.

———'60ª: Note sur l'origine et le mode de formation des canaux périspermiques dans la graine des Marantées. Ann. Sci. Nat., Bot., 4ª Sér., T. XIII, p. 97, 2 pl., 1860.

——— '64: Recherches anatomiques et physiologiques sur la germination. Ann. Sci. Nat., Bot., 5ª Sér., T. II, p. 1, 4 pl., 1864.

Grüss, J., '95: Die Diastase im Pflanzenkörper. Ber. d. d. bot. Ges., Bd. XIII, p. 2, 1 Taf., 1895.

Guignard, L., '82: Recherches sur le sac embryonnaire des Phanérogames angiospermes. Ann. Sci. Nat., Bot., 6ª Sér., T. XIII, p. 136, 5 pl., 1882.

——— ——— '93: Recherches sur le développement de la graine, et en particulier du tégument séminal. Journal de Botanique, T. VII, p. 1, 1893.

HABERLANDT, G., '84: Physiologische Pflanzenanatomie. Leipzig, 1884.

———— '90: Die Kleberschicht des Gras-endosperms als Diastase-ausscheidendes Drüsengewebe. Ber. d. d. bot. Ges., Bd. VIII, p. 40, 1890.

HEGELMAIER, F., '74: Zur Entwickelungsgeschichte monocotyledoner Keime, &c. Bot. Zeitung, Bd. XXXII, p. 631, 2 pl., 1874.

HOFMEISTER, W., '49: Die Entstehung des Embryo der Phanerogamen. 14 Taf., Leipzig, 1849.

———— '61: Neue Beiträge zur Kenntniss der Embryobildung der Phanerogamen. II. Monocotyledonen. Abhandl. d. math.-phys. Kl. d. K. Sächs. Ges. d. Wiss., Bd. VII, p. 631, 25 Taf., 1861.

HOLFERT, J., '90: Die Nährschicht der Samenschalen. Flora, Bd. LXXIII, p. 279, 1890.

KAYSER, G., '93: Beiträge zur Kenntniss der Entwickelungsgeschichte der Samen, &c. Jahrb. f. wiss. Bot., Bd. XXV, p. 79, 4 Taf., 1893.

KLEBS, G., '85: Beiträge zur Morphologie und Biologie der Keimung. Arb. d. bot. Inst. zu Tübingen, Bd. 1, p. 536, 1885.

MEUNIER, A., '90: Les téguments séminaux des Cyclospermées. La Cellule, T. VI, p. 297, 7 pl., 1890.

MIRBEL, C. F. Br., '10: Examen de la division des végétaux en endorhizes et exorhizes. Ann. du Muséum, T. XVI, p. 419, 6 pl., 1810.

———— '15: Elémens de physiologie végétale et de Botanique. Paris, 1815.

MÜLLER, Fr., '83: Einige Nachträge zu Hildebrand's Buche: Die Verbreitungsmittel der Pflanzen. Kosmos, Bd. XIII, p. 275, 1 pl., 1883.

NEES VON ESENBECK, C. G., '31: Uber die Gattungen *Maranta* und *Thalia*. Linnaea, Bd. VI, p. 303, 1831.

OVERHAGE, C., '87: Anatomische Untersuchung und Keimungsgeschichte der Samen von *Canna* und *Musa*, &c. Inaug.-Diss., Erlangen. Ahlen, 1887.

PFEIFFER, A., '91: Die Arillargebilde der Pflanzensamen. Engler's Bot. Jahrb., Bd. XIII, p. 492, 1 Taf., 1891.

PLANCHON, J. E., '45: Développement et caractères des vrais et des faux arilles. Ann. Sci. Nat., Bot., 3e Sér., T. III, p. 275, 2 pl., 1845.

RICHARD, L. C., '11: Analyse Botanique des Embryons endorhizes ou monocotyledonés, &c. Annales du Muséum, T. XVII, p. 223, 6 pl., 1811.

———— '31: De Musaceis commentatio. Nova Acta Acad. C. L.-C. N. C., Bd. XV, P. II, Suppl., 12 pl., 1831.

SCHLEIDEN, M. J., '39: Ueber Bildung des Eichens und Entstehung des Embryos bei den Phanerogamen. Nova Acta, &c., Bd. XIX, P. I, p. 27, 6 Taf., 1839.

———— und VOGEL, Th., '42: Ueber das Albumen, &c., Nova Acta, &c., Bd. XIX, Pt. II, p. 51, 6 Taf., 1842.

STRASBURGER, E., '80: Zellbildung und Zelltheilung. 3e Aufl. Jena, 1880.

TSCHIRCH, A., '90: Die Saugorgane der Scitamineen-Samen. Sitzb. d. K. Akad. d. Wiss. zu Berlin, Jahrg. 1890, I, p. 131. Also, in greater detail, in Ann. Jard. Bot. de Buitenzorg, T. IX, p. 143, 5 Taf., 1891.

WITTMACK, L., '68: *Musa Ensete*. Ein Beitrag zur Kenntniss der Bananen. Linnaea, Bd. XXXV (N. F., Bd. I), p. 209, 1 Taf., 1868.

EXPLANATION OF FIGURES IN PLATES I–IV.

Illustrating Dr. Humphrey's paper on the development of the seed in the Scitamineae.

List of abbreviations used :—*a.* archesporial cell; *al.* aleurone layer; *ant.* antipodal cells; *ar.* aril; *c.* constriction of funiculus; *ch.* chalaza; *ch. i. i.* chalazal inner integument; *ch. m.* chalazal mass; *e.* egg-cell; *egg.* egg-apparatus; *emb.* embryo; *end.* endocarp; *e. s.* embryo-sac or the cavity developed from it; *e. s. m.* embryo-sac mother-cell; *esp.* endosperm; *esp. n.* endosperm-nucleus; *exoc.* exocarp; *ext.* outer layer of outer integument; *f.* funiculus; *g. l.* germinal lid; *g. s.* germinal slit; *i. i.* inner integument; *int.* inner layer of outer integument; *l. l.* light line; *m.* micropyle; *m. c.* micropylar collar; *m. p.* micropylar pad; *n.* nucellus or nucellar tissue; *n. e.* nucellar epidermis; *nut.* nutrient layer of outer integument; *o. i.* outer integument; *ov. c.* cavity of ovary; *p. c.* perisperm-canal; *per.* pericarp; *psp.* perisperm; *p. t.* pollen-tube; *scl.* sclerotic layer of testa, rudimentary or developed; *scl. p.* sclerotic plug; *st.* stoma; *syn.* synergidae; *t.* testa; *tap.* tapetal cell or cells; *v. b.* vascular bundle.

Note.—All sections pass through the plane of symmetry of ovule or seed, unless otherwise stated.

PLATE I.

Figs. 1–19 a. *Canna indica*, L.

Fig. 1. A young ovule just after the first division of the archesporial cell. ×350.

Fig. 2. A nucellus after the first division of the embryo-sac mother-cell; 1°, the cell cut off by the division. ×260.

Fig. 3. A nucellus after the second division of the embryo-sac mother-cell; 1° and 2°, the two cells cut off. ×260.

Fig. 4. An ovule ready for fertilization, the antipodals not shown. ×100.

Fig. 5. Young seed of ·8 mm. in length, showing at *c* the beginning of the constriction of the funiculus. ×22.

Fig. 6. Young seed 1·5 mm. long, showing upward bending of inner integument. ×22.

Fig. 7. Young seed 2 mm. long. ×22.

Fig. 8. Young seed 3 mm. long. ×22.

Fig. 9. Contents of embryo-sac of a young seed 3 mm. long. ×200.

Fig. 10. Part of micropylar end of a seed of 4 mm. ×22.

Fig. 11. Region of the germinal slit of a young seed of 4 mm. ×200.

Fig. 12. Section of ripe seed, with embryo removed. ×2.

Fig. 13. Micropylar end of testa, showing kite-shaped area free of stomata. ×3.

Fig. 14. Part of micropylar end of ripe seed, showing distribution of tissues in the testa. ×22.

Fig. 15. Structure of coat of a young seed 2·5 mm. long. ×200.

Fig. 15 a. Details of outer layers of Fig. 15. ×530.

Fig. 16. Structure of coat of a young seed 3·5 mm. long. × 200.
Fig. 16 a. Details of outer layers of Fig. 16. × 530.
Fig. 17. Structure of coat of seed 6 mm. long. × 200.
Fig. 17 a. Details of outer layer of Fig. 17. × 530.
Fig. 18. Structure of testa of full-grown, but unripe, seed. × 200.
Fig. 18 a. Details of outer layer of Fig. 18. × 530.
Fig. 19. Structure of testa of ripe seed. × 200.
Fig. 19 a. Details of outer layer. × 530.

PLATE II.

Figs. 20–24. *Canna indica*, L.

Fig. 20 a. Surface view of a stoma from the surface of a young seed 3·5 mm. long. × 530.
Fig. 20 b. A vertical section of the same. × 530.
Fig. 21. Vertical section of a stoma in the ripe testa. × 530.
Fig. 22. Cells of the endosperm-layer from a seed not yet quite ripe. × 530.
Fig. 23. Cells of the endosperm-layer from a ripe seed. × 530.
Fig. 24. Section through endosperm-layer and already emptied perisperm-cells from a germinating seed. × 530.

Figs. 25–31. *Thalia dealbata*, Fraser.

Fig. 25. A young ovule. × 75.
Fig. 26. An ovule a little before fertilization. × 60.
Fig. 27. Embryo-sac of an ovule ready for fertilization. × 530.
Fig. 28. Section through the integuments of such an ovule. × 200.
Fig. 29. Young seed, with projection into this median plane of beginning of perisperm-canal. × 30.
Fig. 30. Fully grown but unripe seed enclosed in the pericarp. × 4.
p. c., the projection into this plane of the perisperm-canals, which lie respectively above and below the plane of the section; the same is true for Fig. 29.
Fig. 31. Structure of testa of nearly ripe seed. × 200.

Fig. 32. *Calathea densa*, Regel, a well-developed ovule. × 75.
Fig. 32 A. *Phrynium capitatum*, Willd., section of a ripe seed, with embryo removed. × 2.

Figs. 33–37. *Amomum elongatum* (Teijsm. et Binn.).

Fig. 33. Very young ovules. × 200.
Fig. 34. An older ovule. × 75.
Fig. 35. Structure of integuments of developed ovule. × 200.
Fig. 36. Section of ripe seed. × 6.
Fig. 37. Structure of part of micropylar end of ripe seed. × 38.

Figs. 38–40. *Alpinia mutica*, Roxb.

Fig. 38. A young ovule. × 75.
Fig. 39. Section of a nearly ripe seed at right angles to the diaphragm, *dm.* × 5.
Fig. 40. Section of a similar seed in the plane of the diaphragm, *dm.* × 5.

PLATE III.

Figs. 41–49. *Costus* sp.

Fig. 41. A very young ovule. × 260.
Fig. 42. An older ovule. × 350.
Fig. 43. A still older stage. × 260.
Fig. 44. A well-formed ovule. × 100.
Fig. 45. An ovule at about the time of fertilization. × 75.
Fig. 46. A young seed of about four-fifths of its final length. × 20.
Fig. 47. Structure of coat of a seed slightly younger than that shown in Fig. 46. × 530.
Fig. 48. Section of ripe seed. × 20.
Fig. 49. Structure of region indicated at '49' on Fig. 48. × 100.
Fig. 49 a. Details of a part of Fig. 49. × 260.

Fig. 50. *Musa* sp., an ovule. × 38.
Fig. 50 a. A nearly or quite full-grown, but unripe, seed. × 10.

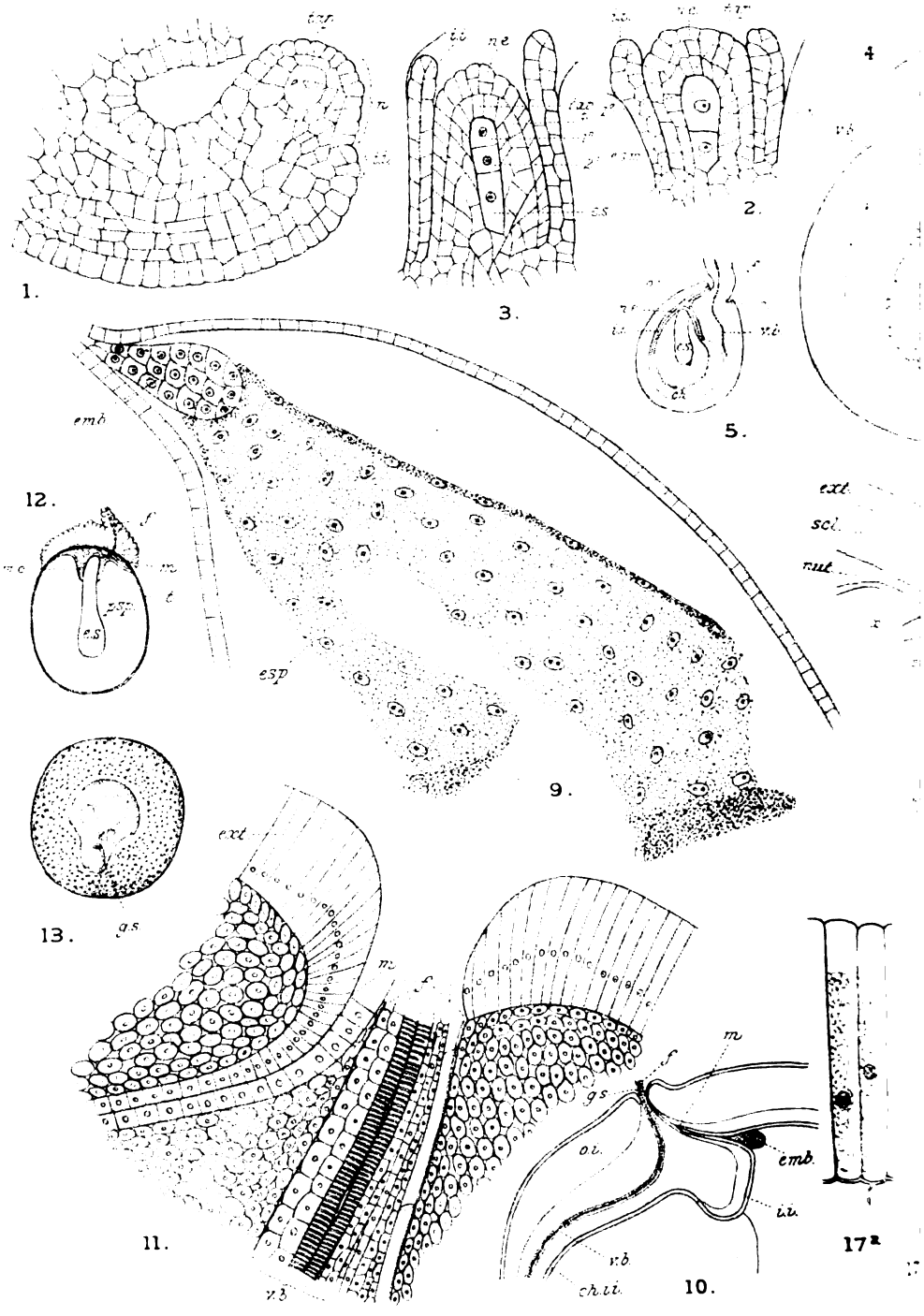
Figs. 51–56. *Strelitzia Reginae*, Ait.

Fig. 51. A young ovule. × 38.
Fig. 52. The micropylar half of an ovule at the time of fertilization. × 30.
Fig. 53. Micropylar half of a full-grown, but unripe, seed. × 10.
Fig. 54. Structure of coat and body of seed shown in last figure. × 75.
Fig. 55. Section of ripe seed with embryo removed. × 3.
Fig. 56. Structure of coat and body of ripe seed. × 100.

PLATE IV.

Figs. 57–70. *Heliconia psittacorum*, L. fil.

Fig. 57. A young ovule. × 530.
Fig. 58. A later stage, showing two cells, 1° and 2°, cut off from the embryo-sac mother-cell. × 200.
Fig. 59. A still later stage, showing four cells derived from the embryo-sac mother-cell. × 100.
Fig. 60. A similar stage to the last, showing *five* cells in the series. × 100.
Fig. 61. An older ovule, with developing embryo-sac. × 200.
Fig. 62. Section through a loculus of the ovary with its ovule, at the time of fertilization. × 10.
Fig. 63. The ovule shown in the last figure. × 40.
Fig. 64. Loculus of an ovary, with young seed. × 10.
Fig. 65. Structure of seed coat and endocarp at stage of Fig. 64. × 260.
Fig. 66. Micropylar end of older seed. × 75.
Fig. 67. Structure of seed and endocarp at stage of Fig. 66. × 260.
Fig. 68. Section of a full-grown and nearly ripe fruit. × 10.
Fig. 69. Section of a ripe fruit. × 10.
Fig. 70. Structure of ripe seed and endocarp. × 260.



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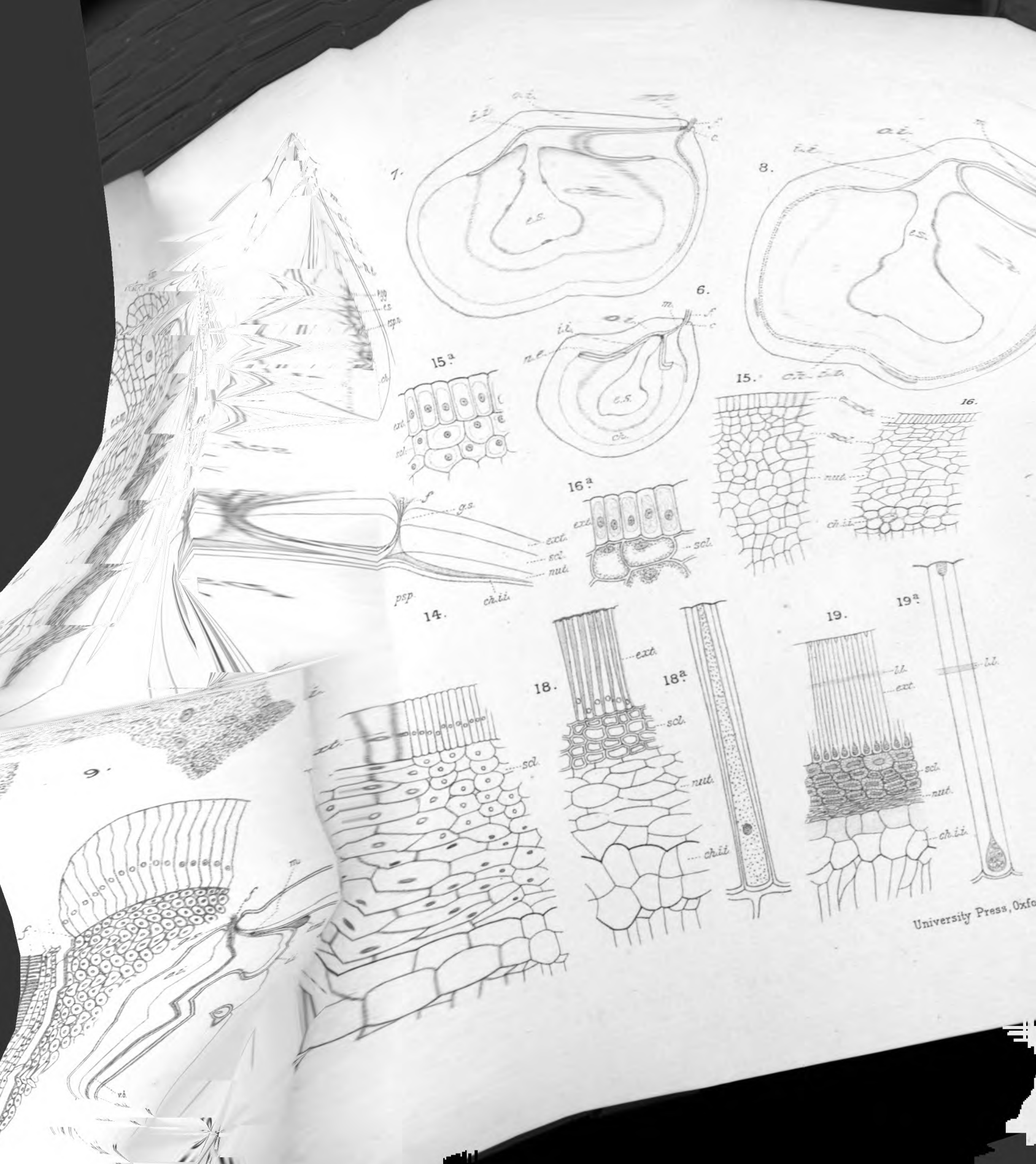

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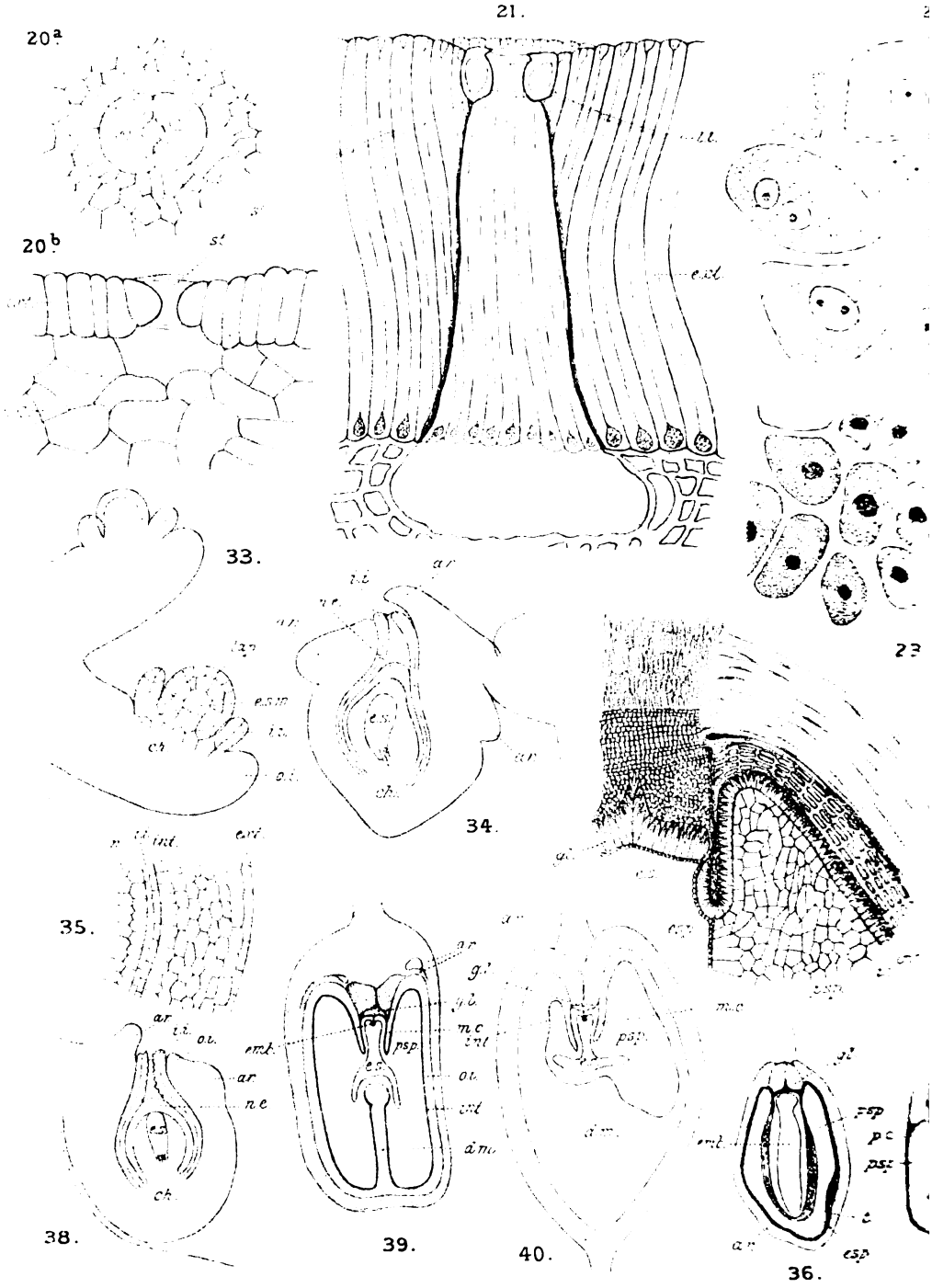

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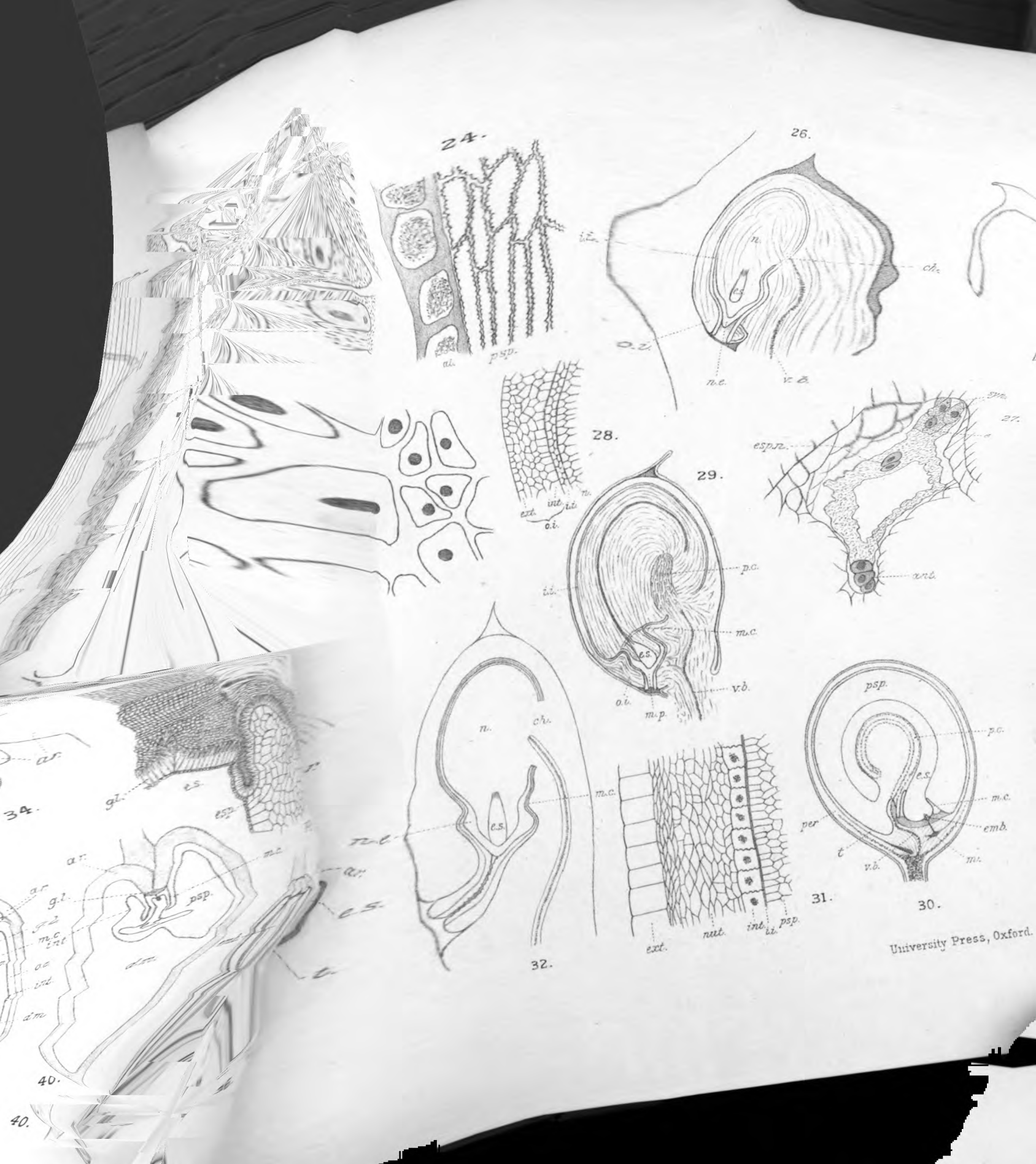

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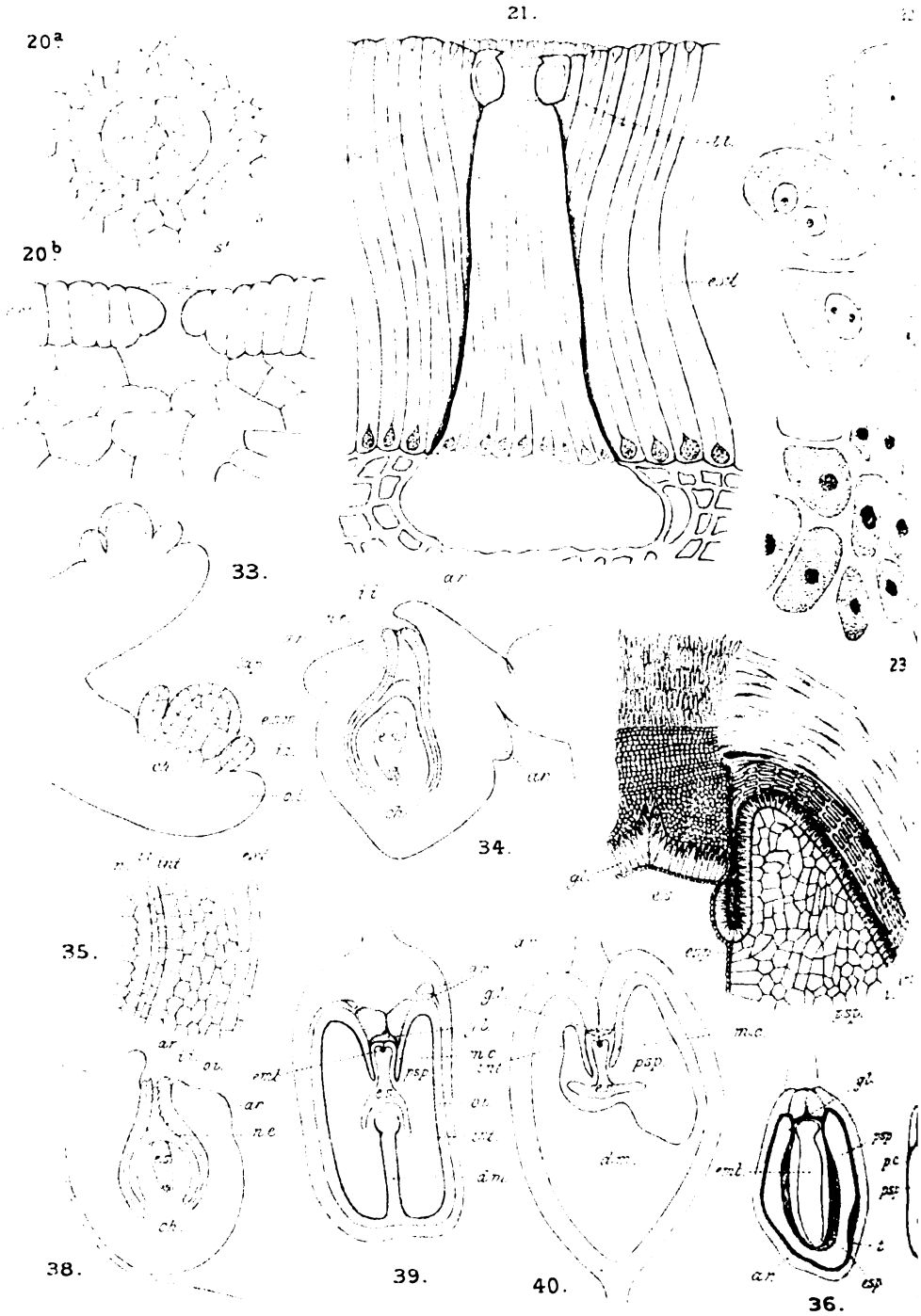

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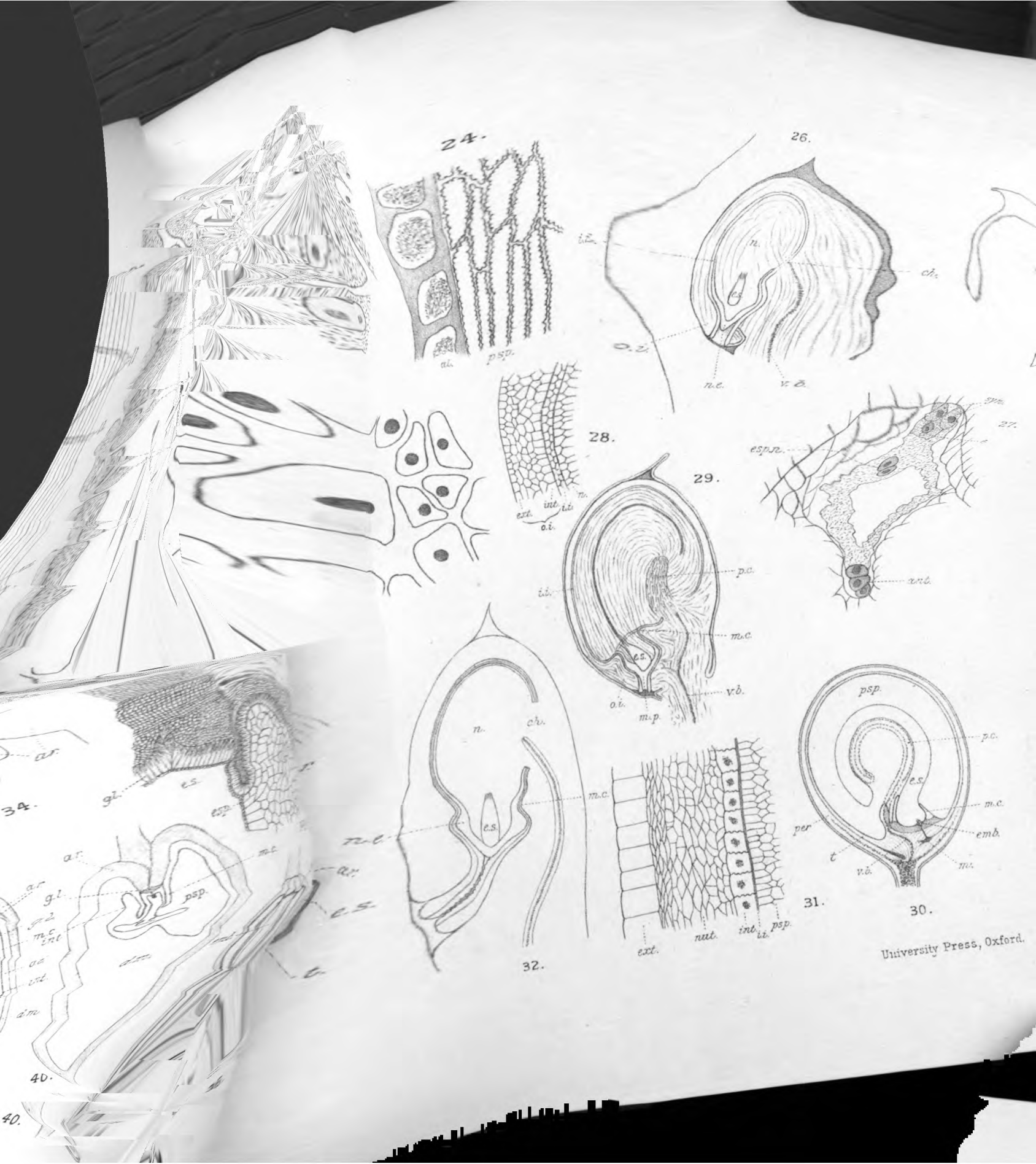

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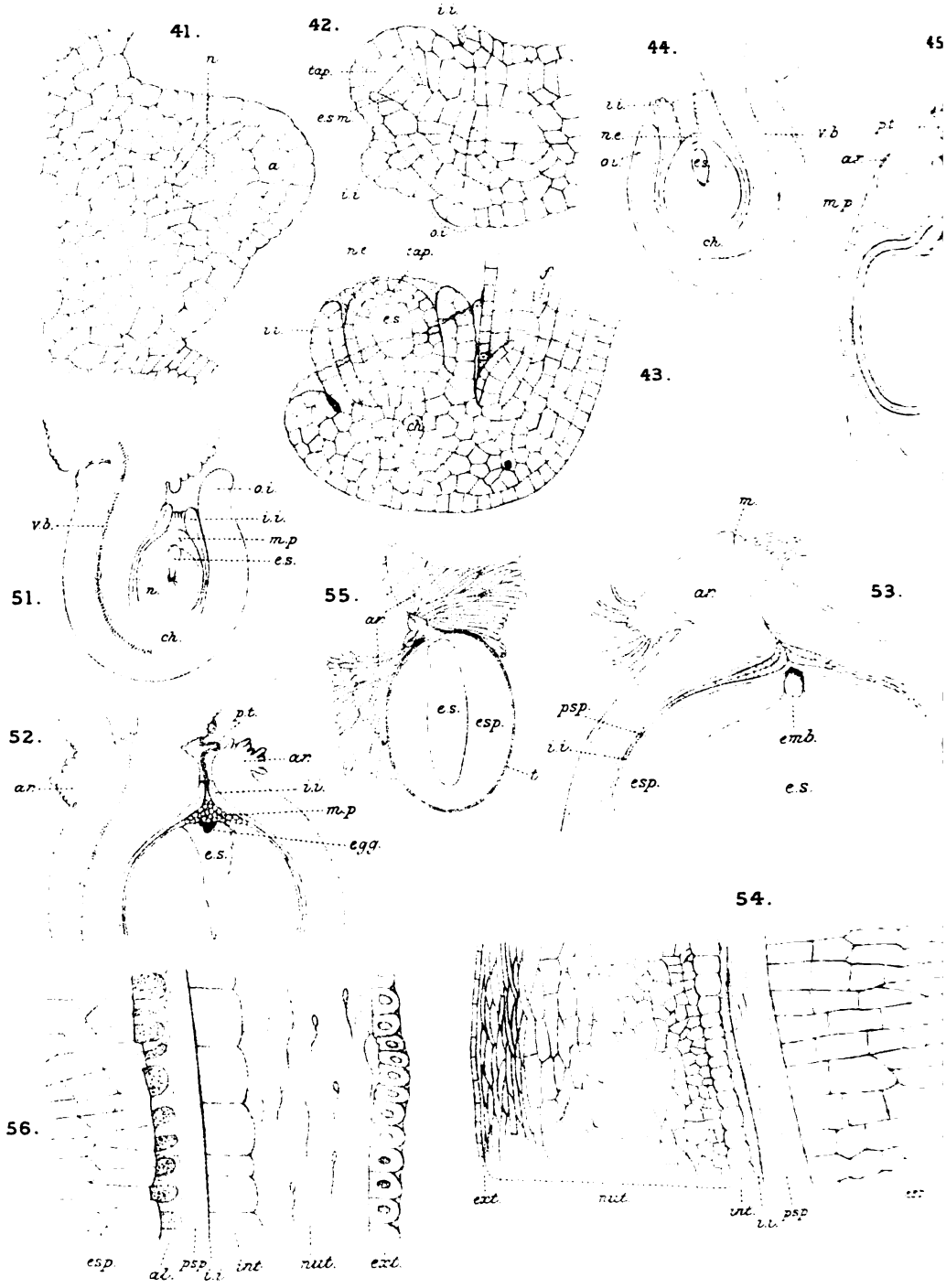

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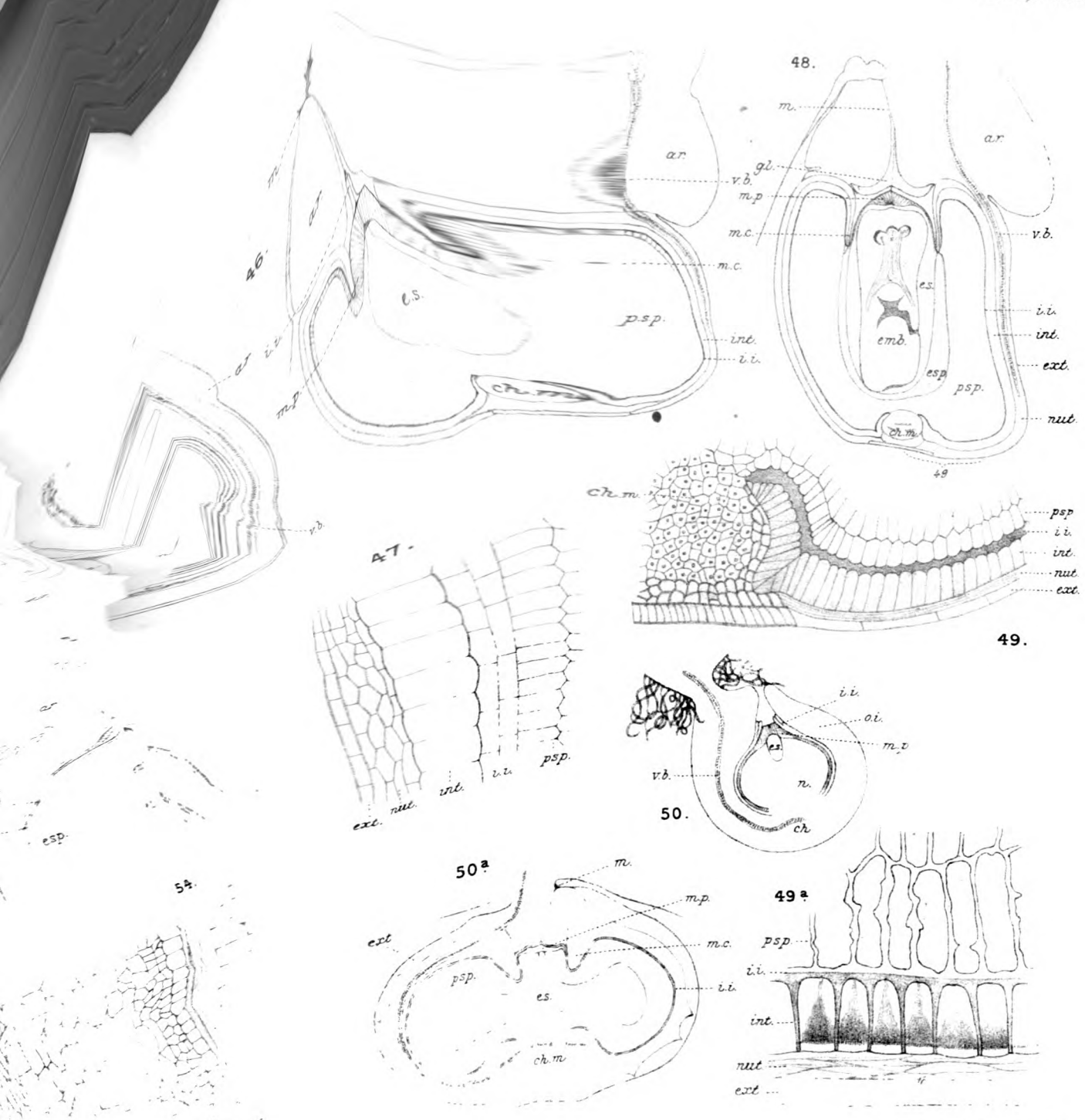

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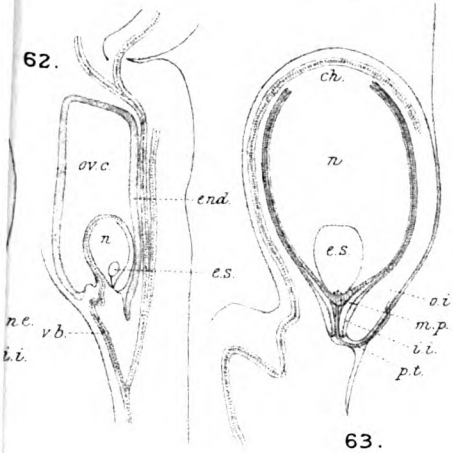

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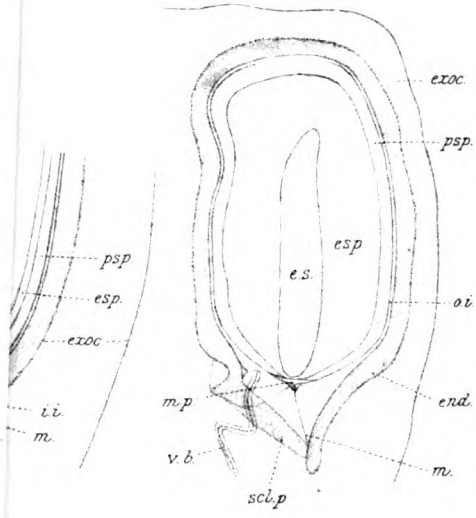

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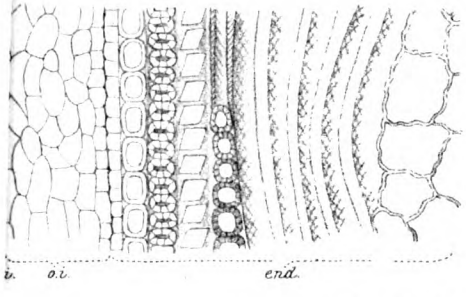

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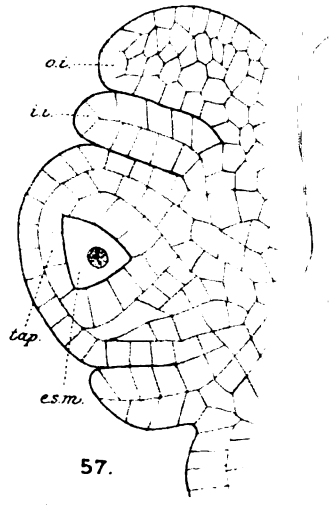

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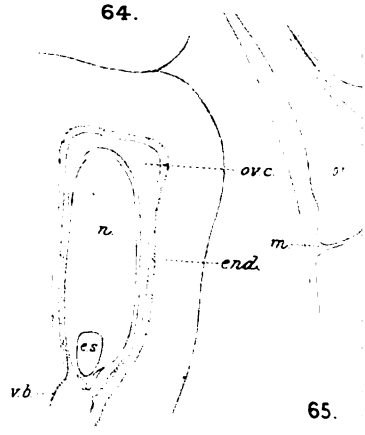

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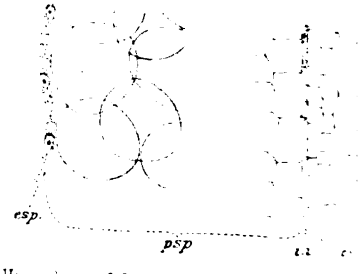

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SEEDS

Nematophyton Ortoni, n. sp.

BY

D. P. PENHALLOW,

McGill University, Montreal.

With Plate V.

SINCE my last general summary of the genus *Nematophyton*[1], two new species from Europe have been recorded, one by Mr. C. A. Barber from the Tymawr quarry, near Cardiff, Wales, and named by him *N. Storriei*[2]; the other from the shales in the neighbourhood of Gräfrath on the lower Rhine, and designated by Count Solms-Laubach *Nematophyton dechenianum*[3].

I have now to describe a third, which differs in very important respects from all previously recorded species, from the middle or upper division of the great Ohio shales. This specimen was obtained by Professor Edward Orton, of the Geological Survey of Ohio, from whom the facts relating to its origin have been obtained, and after whom I would name it *Nematophyton Ortoni.*

[1] Notes on Devonian Plants. Trans. R. Soc. Can. VII. iv. 19.

[2] *Nematophycus Storriei*, Ann. Bot. VI. 329.

[3] Ueber devonische Pflanzenreste aus den Lenneschiefern der Gegend von Gräfrath am Niederrhein. Jahrbuch der königl. preuss. geologischen Landesanstalt. 1894, 67.

[Annals of Botany, Vol. X. No. XXXVII. March, 1896.]

The hand-specimen transmitted to us for examination is 15 cm. high and 14 cm. broad at the base, from which it gradually tapers upward in such a way as to suggest that it must be the base of a stem. This idea is strengthened by the presence of a number of basal and lateral processes with their outward terminations presenting fractured surfaces, showing that they represent the bases of more or less prolonged organs such as roots. Between these processes are rounded indentations, such as may be supposed to have been made by large pebbles. In fact, to those who are familiar with the branching base of a Laminarian stipe, the resemblance between the two is very striking. But Prof. Orton informs me that this specimen was originally part of a very much larger one which was broken in getting it out of the shale. The largest fragment thus obtained measured about 20 inches long and 8 to 10 inches wide. A photograph of it shows the surface to be irregularly indented with several processes, the whole presenting the general aspect of the smaller specimen taken from it. It is clear then that the original plant must have been one of rather large dimensions, on the scale of a tree, and the general external characters of the specimens justify the view that they represent the base of a stem or stipe at the point whence the roots issue.

Internally the specimen is highly silicified throughout, and shows no evidence whatever of concentric structure. Externally there are limited areas covered with a friable, carbonaceous film which, in places, attains a thickness of about 1·5 mm., but for the most part it is very thin. It in all probability represents the carbonised residue of the cortical tissue, although microscopical examination fails to disclose any definite structure. We can therefore only assume that it represents cortex, a view, however, which finds its justification in our general knowledge of the alterations in such structures under similar conditions.

The first sections examined were received from Prof. Orton, but they got badly broken in transit, in consequence of which, and also for the purpose of ascertaining the relation of struc-

ture to special locations, a new series of sections was prepared at the Peter Redpath Museum. It was found upon examination that there were no material differences of structure exhibited by the two sets, so that no special reference to them is required. The structure was found to be most beautifully preserved, and in this respect it is comparable with the Gaspé specimens of *N. Logani.*

In transverse section the structure is seen to consist of numerous round, thick-walled, and rather widely separated cells of rather uniform size, the larger having a diameter of about 67 μ. Between these cells others are to be seen running more or less transversely at various angles, and therefore interlacing with the first or longitudinal series. The spaces between the cells are occupied by small crystals of silica and a limited amount of carbonaceous matter, which shows no disposition indicative of structure. So far very searching examinations have failed to disclose anything of the nature of smaller hyphae such as distinguish the structure of *N. Logani*, *N. crassum*, and *N. laxum*, and it is impossible to say if such were at any time present, though the presence of carbonaceous matter among the siliceous crystals would seem to suggest the possibility of hyphae, or at least of some structure, having been present, since it has already been shown in the case of *N. Logani* and *N. crassum*, that the carbonaceous matter of the original structure often becomes distributed in such ways through the influence of crystallization [1].

There is no evidence whatever of radial spaces such as were found in *N. Logani.* But there are rather numerous isodiametric areas, about 0·30 mm. broad, occupied entirely (Fig. 3) by masses of very narrow and densely interlacing hyphae, having a diameter of 4·8 μ. In the sections so far submitted to examination, the structure of these hyphae has, in most cases, been destroyed by crystallization, and in the few instances where the structure was intact it was not possible to ascertain if they were septate, though the occurrence of

[1] M. Micr. Jnl. X. 69, 70. Trans. R. Soc. Can. VII. iv. 23; Proc. U. S. Nat. Mus. XVI. 115.

septa in the similar hyphae of *N. Logani*, as determined by Mr. Barber[1], would seem to indicate the possibility of their presence in this case. In no instance do these hyphae appear to cling to the walls of the large tubes of the medulla as in *N. Storriei*, but they fill isolated cavities which, for want of a better term, I may designate as the medullary spots. These spots exhibit the same general form and dimensions in all planes of section, and on smoothly cut surfaces of the hand-specimen they often appear as small cavities about 1·0 mm. broad, frequently containing minute crystals. We thus have evidence that these spots are not always occupied by small hyphae, a fact which suggests either (1) that the spaces are normal, and the hyphae intrusive growths, or (2) that the small hyphae are normal, and in some cases have been removed by decay or other causes. Which of these views is correct our material does not permit me to say; but the view advanced by Mr. Barber, and strengthened by our knowledge of similar openings in the various known species of *Nematophyton*, that these spaces have some connexion with the aeration of the plant[2], would seem to offer a reasonable explanation of their occurrence. It may also be pointed out that the most marked alterations of structure, through decay (?) and crystallization, to be met with in the present species are found in the hyphae of these areas. With respect to the occurrence of these medullary spots, the present species approaches somewhat closely to *N. crassum*.

In longitudinal section it is wholly unnecessary to distinguish between the radial and tangential planes, since the structure presents the same aspects in each case. The structure consists of broad, tubular cells running in a direction generally parallel to the axis of growth, together with others less numerous, but yet in large numbers, traversing the stem in all directions, so that unlike the species hitherto described, there is a want of definiteness in direction. Occasionally these cells are exposed to a great length, but more commonly the plane of section cuts them off at frequent intervals so

[1] Ann. Bot. VI. 333. [2] Ann. Bot. VI. 337.

that only short fragments appear (Figs. 2 and 3). A marked peculiarity of the cells in this plant is the frequency with which local expansions of the lumen occur. These we can only regard as representing the trumpet-hyphae and situations of sieve-plates so common in the Laminarieae. Although in the majority of cases no sieve-plate could be observed, in a few instances the fact of such structures having been present was quite obvious (Figs. 2 and 3). One of the trumpet-hyphae is shown on a much larger scale in Fig. 5. I have had no very good opportunity of instituting a comparison between these structures and the forms occurring in the larger species of the Laminarieae. My chief comparison, therefore, has been with the forms common to the North Atlantic coast; but through the kindness of Dr. W. G. Farlow, it has been possible to take into consideration *Macrocystis pyrifera*[1]. Although differing in detail, the general character of these structures in *Nematophyton* and *Macrocystis* is so similar as to suggest the belief that our fossil is related to those modern types of seaweeds of which *Macrocystis* is an example. As in the transverse section, no small hyphae are to be found between the large cells, but the latter are seen to branch somewhat frequently and always, so far as determined from the present material, in the immediate vicinity of a medullary spot (Fig. 4).

From the details thus outlined it is clear that the plant is an Alga, and of an alliance with the Laminarias. Having regard to the general character of the stem-structure, it is evident from our specimens that the cortical layer was relatively thin, the medulla strongly predominating, and in these respects the stem presents features which are well represented by *Laminaria digitata*.

This species differs from all others so far known, in the very loose character of the medulla, a feature which may be characteristic of the species as a whole, but which may belong more particularly to certain regions of the plant, and this

[1] In this connexion comparison may be made with the various forms of trumpet-hyphae in *Macrocystis*, as figured by Prof. F. W. Oliver in Ann. of Bot. I. 95.

view gains strength from the fact that our sections were apparently taken from one of the hapteres near its junction with the principal axis.

In his article on *N. Storriei*, Mr. Barber finds it difficult to agree with my views respecting the organic connexion between the large cells of the medulla and the small hyphae, basing his objections upon examinations of *N. Logani* and *N. Storriei*; but in view of the evidence at hand, it is impossible for me to accept the modifications he suggests[1]. Evidently when his article was written he had not seen my second paper on *Nematophyton*[2], in which five species are described. In speaking of *N. crassum*[3], I then made use of the following description :—

'The most significant fact so far observed, consists in the discovery of a distinctly branching system, similar in its general character to that of *N. Logani*, though differing from it in some important respects. In one case I found a branch projecting from the side of a large cell, with a diameter of 5·8 μ and a length to the point where cut off of about 35 μ. Two other branches near together were each 4·6 μ; two more were 2·3 μ and 4·6 μ; another 6·9 μ in diameter . . . It was therefore clear that the larger cells of this plant branch into a secondary plexus as in *N. Logani*, and as all of the instances in which the branches were seen to emanate from the larger cells occurred in the open tracts above described, it would appear that these latter serve as the special regions in which branching is effected.'

We have here, then, the fact that in *N. crassum* the large cells do branch into small filaments of the same general diameter as the small hyphae of the spaces, and that such branching takes place where these hyphae are most abundant. If this be taken in connexion with Mr. Barber's admission that the branching is most frequent in the region of the spaces, it will be seen that there are good grounds for denying his contention with respect to *N. Logani*, *N. crassum*, and *N. laxum*, while the presumption would be in favour of

[1] Ann. of Bot. VI. 335. [2] Trans. R. Soc. Can. VII. iv. 19.
[3] L. c., p. 22.

regarding similar structural conditions to exist in the other species where the evidence is not so well defined. Nevertheless, it must be remembered that the genus *Nematophyton*, as we now know it, is made up of several apparently distinct species of whose entire structure we know but little: and it is quite within the range of possibility—as is even now suggested by the striking structural differences presented by *N. Ortoni*—that when we are able to reconstruct the entire organism in each case, it may be found that more than one genus is represented, or that some of those which now appear distinct, may in reality be different parts of the same species.

Our present knowledge of the genus *Nematophyton* shows that it embraces what appear to be eight distinct species as follows:—

1. **N. Logani**, Dn. Lower Erian of Gaspé; Silurian (Upper Ludlow) of England and Silurian (Cap Bon Ami) of New Brunswick. (*Dawson.*)
2. **N. Hicksii** (Eth.), Dn. Denbighshire grit (Silurian) of Wales. (*Hicks.*)
3. **N. crassum** (Dn.), Penh. Middle Erian of Gaspé (*Bell*); Hamilton Group (Middle Erian) of New York. (*Clarke* and *Prosser.*)
4. **N. laxum** (Dn.), Penh. Lower Erian of Gaspé. (*Bell.*)
5. **N. tenue** (Dn.), Penh. Lower Erian of Gaspé. (*Bell.*)
6. **N. Storriei**, Barb. Silurian (Wenlock Age) of Cardiff, Wales. (*Storrie.*)
7. **N. dechenianum**, Solms-Laub. Upper Devonian of Gräfrath, Germany. (*Solms-Laubach.*)
8. **N. Ortoni**, n. sp. Upper Erian of Ohio. (*Orton.*)

EXPLANATION OF FIGURES IN PLATE V.

Illustrating Prof. Penhallow's paper on *Nematophyton Ortoni*, n. sp.

Description of Figures.

Fig. 1. Transverse section showing the general character of the structure. ×45.

Fig. 2. Longitudinal section showing the general character of the medulla, with trumpet-hyphae, and on the extreme left of the field a portion of a medullary spot. ×45.

Fig. 3. Longitudinal section showing the occasional exposure of the tubular cells for a great length, trumpet-hyphae, and two medullary spots on the left. ×45.

Fig. 4. Transverse section showing a cell of the medulla with, at the centre of the figure, three branches. The darker area of the field on the upper side is the side of a medullary spot. ×170.

Fig. 5. Longitudinal section showing trumpet-hyphae at centre and left side, with crystals of silica occupying the spaces between the large tubular cells. ×170.

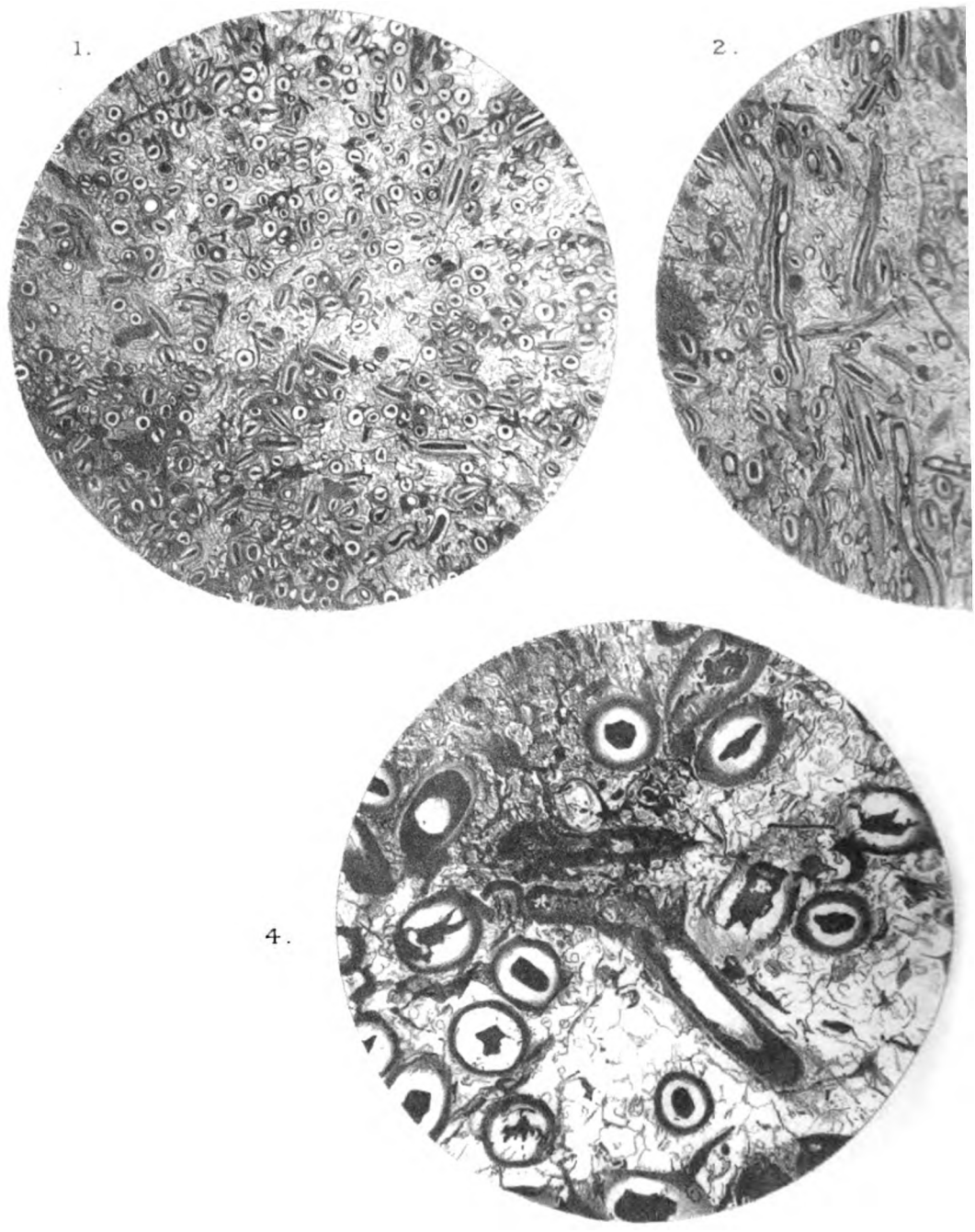

Photo. Photoc.

PENHALLOW. — NEMATOPHYTON ORTONI.

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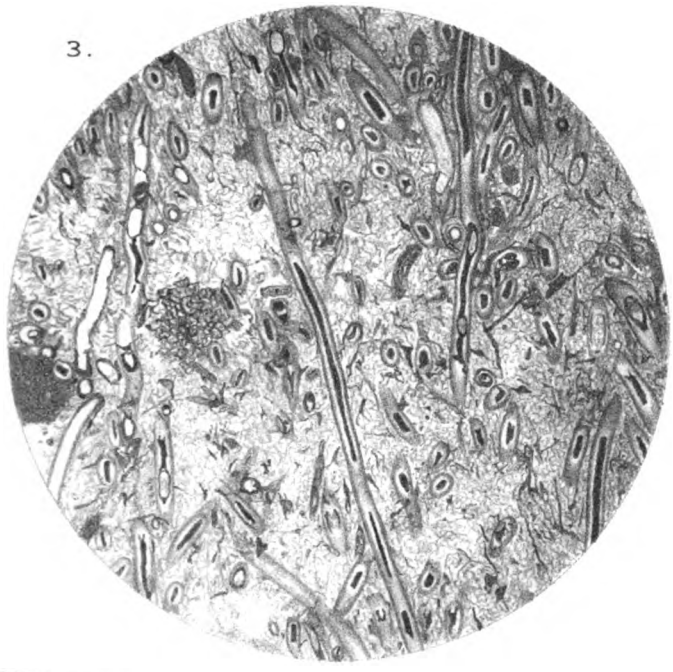

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University Press, Oxford.

The Fertilization of Batrachospermum [1].

BY

BRADLEY MOORE DAVIS.

With Plates VI and VII.

DURING the course of his studies at Harvard University, the writer had the opportunity of examining some very excellent material of what appeared to be a winter-form of *Batrachospermum moniliforme*, Roth. As the conditions were particularly favourable in this plant for the study of the nuclei in the procarps and antherozoids before and after fertilization, the writer began a series of observations upon the subject. The results were so surprising that he supplemented this study with the examination of two other species of the same genus, *B. coerulescens*, Sirdt. and *B. Boryanum*, Sirdt.

Certain peculiar positions of the nuclei in the cells of the procarps and antherozoids at the time of fertilization and after the development of the cystocarp, led the author to think at one time that perhaps the cystocarp developed apogamously. These puzzling conditions were supplemented by the fact that in a certain proportion of the preparations, cystocarps, in various stages of development, were found which bore trichogynes lacking the usual accompanying fused antherozoid. In many cases it was not easy to believe that the antherozoid

[1] Contribution from the Cryptogamic Laboratory of Harvard University, No. 31, prepared under the direction of Dr. W. G. Farlow.

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had been torn off in the manipulation of the specimens. This interesting question of possible apogamy led the writer to try some experiments upon living plants that had best be considered at the outset.

In nature, the filaments of the *Batrachospermum*-plants, being attached to stones and other objects usually in running water, float out in the direction of the current and wave slightly to and fro with the varying movements of the stream. As the plants grow in tufts, the separate filaments necessarily touch and scrape against one another when their positions change in the flowing water: hence it seems quite possible that the antherozoids of one plant or filament might readily be brushed off upon the trichogynes of another as the branches move from side to side. It is difficult to understand how the antherozoids could be brought in such profusion and applied to the trichogynes by swiftly-moving water, and the above explanation of the process of fertilization appears very plausible to the author.

To determine experimentally whether contact of antherozoids with trichogynes is absolutely necessary for the production of fruit, it is necessary to grow plants under such conditions that the possibility of fertilization either by direct contact of the filaments or by currents of water is removed. The normal conditions of environment, temperature, light, &c., must at the same time be strictly adhered to, in order that the experiment may be conclusive. *Batrachospermum Boryanum* is a strictly dioecious species; and one may frequently find plants of *B. coerulescens* which bear very few or no antherozoids: but this special form of *B. moniliforme* is monoecious. It is an easy matter to select female plants of *B. Boryanum* and *B. ccerulescens*, and to clear the stone upon which they grow of all male plants. However, it is not easy to grow such female plants isolated in aquaria, because it is so difficult to imitate the normal surroundings of *Batrachospermum*-plants as regards the temperature of the water, for they frequently grow in ice-cold streams: the plants do not thrive when removed from their running brooks: But the writer believes

that he has satisfied the conditions (isolation of female plants and normal environment) necessary to answer the problem stated at the beginning of the paragraph, in the following manner.

Some stones, upon which were female plants of *B. Boryanum* and *B. coerulescens*, carefully freed from antheridial specimens, were placed in large glass jars. The jars were fitted with covers having a few holes punched in them, and were then sunk in the brook in the same situations from which the plants were collected. The conditions as regards light and temperature of the water were therefore the same, for the prepared plants and those growing wild around them. The holes in the covers allowed a constant slow interchange of water, but the swiftness of the current was greatly abated; in fact, the plants were growing in almost perfectly still water. Of course the chances of antherozoids being brought to the trichogynes by the currents of water were thus greatly diminished. Jars containing plants of each of the three species were placed in the stream in the middle of December, and were taken out at different times and examined, the last having been in the stream two and one-half months. The experiments give very positive data. In the first place the plants in the jars appeared perfectly healthy. Two to three centimetres of new growth were added to each branch. The habit was not materially different, except that in some cases the new branches were not so stout as the old growth, the tendency being to produce long thin structures rather than short thick ones. The colour of the plants remained the same, and the cells of the tissue did not differ in any marked manner from those of plants under normal conditions.

An abundance of procarps were produced in the situations peculiar to each species, and seemingly in the usual quantity. Almost all the trichogynes of *B. Boryanum* and *B. coerulescens* were free from antherozoids, and in each case there was no indication of any tendency to produce fruit apogamously. The trichogynes did not wither, but remained attached to the procarps in the usual manner, so that as one examined

a branch one procarp followed another, each identical in appearance with its neighbour. In the case of *B. moniliforme*, the monoecious species, a very fair proportion of the trichogynes were free from antherozoids, perhaps because the quiet water in the jar prevented the filaments of the plants from rubbing together. Those procarps whose trichogynes lacked antherozoids never developed fruit, while neighbouring trichogynes with antherozoids fastened to them were attached to cystocarps in various stages of development, some with ripe spores. The evidence seemed almost conclusive that fusion of an antherozoid with the trichogyne was absolutely necessary for the production of fruit. As the reader proceeds, he will understand why it is desirable to dwell upon this point, even though it be what has always been considered a settled fact.

The tissues of *Batrachospermum* may be most readily studied from crushed-out preparations, which are very easily made because of the flexible character of the filaments and the amount of mucilaginous matter in the frond. It has been the writer's experience that absolute alcohol is the most satisfactory fixing agent, for it preserves the mucilage, which is very helpful in the further manipulation of the material. Chromic acid dissolves the mucilage. It is also possible that chromic acid may weaken the attachment of the antherozoids upon the trichogynes, and allow them to be more easily torn off in manipulation; for it was in material fixed with this reagent that the writer found many specimens of cystocarps whose trichogynes were without these accompanying structures. Of the many stains tried, the haematoxylin formulae gave the best results, and the most satisfactory of these were Mayer's haemalum and acid haemalum, the latter being the more precise stain. Böhmer's haematoxylin is also good. There is little choice between the common fixing agents, and absolute alcohol was employed for the reason before stated, that material killed with this fluid was more easily crushed out because of the mucilage contained in the frond. High powers of the microscope were necessary throughout the investigation, and the writer used the Zeiss 4 mm. apochro-

matic objective with compensation ocular No. 12 (magnification 750 diameters) even in ordinary work, and relied upon a Leitz $\frac{1}{12}$ oil immersion lens for the more minute observations.

The paper is so divided that the accounts of the three species contain but a bare statement of facts; the consideration of the bearing of the results upon the present accepted view of the structure and fertilization of the procarp in the genus being reserved for the concluding portion of the paper.

BATRACHOSPERMUM MONILIFORME, *Roth.*

The plant which we have identified as a winter-form of *B. moniliforme*, grew very abundantly in a quietly-flowing mill-race, covering the stones and dead branches along the sides and bottom of the stream with a thick growth an inch or more in height. The plant matures in mid-winter, and is then almost the sole living occupant of the stream. The material from which the figures are drawn was gathered in February, when the plant was at its best.

The trichogynes are club-shaped structures at the ends of short branches, which extend usually about half the distance from the central axis of the plant to the periphery. At the outset it was immediately apparent that each trichogyne was not merely a swollen prolongation of a cell, the carpogonium[1], which contained the nucleus. There was not only a distinct nucleus in the carpogonium, but also an unmistakable nucleus in the trichogyne, staining with haematoxylin as a dark blue body, quite homogeneous in structure. The

[1] It has been a serious question to the writer what nomenclature of the organs of fructification in *Batrachospermum* he should adopt. The term carpogonium was applied by Schmitz (Untersuchungen über die Befruchtung der Florideen) to a particular cell because of its resemblance to the oogonium of certain Chlorophyceae, notably *Coleochaete*. The trichogyne was considered as an extension of this cell specialized for the reception of the antherozoid. The writer's observations have led him to a different view of the structure of the trichogyne, but to avoid confusion he has used the term carpogonium in the strict sense suggested by Schmitz, applying it to the particular cell that bears the trichogyne and gives rise to the fertile filaments, the trichophore in the terminology of Bornet and Thuret.

protoplasm directly around the nucleus usually stains light blue, and the remaining cytoplasm is coloured more or less deeply, depending upon certain structures which will be considered later. The cytoplasm in the carpogonium is often very dense, and is apt to stain deeply, but the very darkly-stained nucleus is usually prominent by virtue of its central position.

The very earliest beginnings of trichogynes cannot be readily distinguished from the tips of ordinary vegetative branches; but a point is soon reached in their development when the form clearly designates the character of the structure. The terminal cell of a branch begins to extend first as a little process, which gradually swells and finally assumes the size and shape of the adult trichogyne. The cell from which the trichogyne springs is of course the carpogonium. It contains a somewhat irregularly lobed chromatophore that lies in the layer of protoplasm next the cell-wall. The chromatophore fills up a large part of the cell, and is very apt to be massed at the end from which the trichogyne arises. When the young trichogyne begins to extend from the carpogonium cell, some of the chromatophore of the latter is apparently drawn up into it. At all events, one finds in the basal portion of young trichogynes, and as a rule also of mature individuals, an irregularly-shaped body that is clearly an extension of the chromatophore of the carpogonium. A glance at Fig. 2, a young trichogyne drawn from living material, will make clearer what has just been described. The shaded portion of the carpogonium (lettered *c*) is the chromatophore, coloured a light green in this species, and all the rest of the cell is quite hyaline but for a few granules scattered here and there, and perhaps one or more vacuoles. It will be seen that the chromatophore of the carpogonium runs up into the trichogyne in the form of an irregular extension. The colour of this extension in the base of the trichogyne is often identical with that of the portion in the carpogonium, but in the upper part of the trichogyne the tint is usually very much lighter. The shapes assumed are very

various, and sometimes the body extends perhaps only a third the length of the trichogyne, sometimes almost to the very end. There are usually one or two rather prominent lobes. Such is the appearance of this body in the young trichogyne, and, the writer, in studying the development of the latter, came to the conclusion that it was truly a portion of the chromatophore of the carpogonium, and deserved to be mentioned as a definite structure in the trichogyne. We have said that the chromatophore in the trichogyne is very variable in its shape, and also in its position, for it lies now at the base of the trichogyne, again along the sides, and sometimes near the top. The boundaries of the chromatophore, which are at first distinct, become less definite as the trichogyne matures and, the outlines grow more irregular. The colour also changes. In the young trichogynes the tint is very similar to the colour of the chromatophore in the carpogonium, that is, a peculiar light green shade that sometimes has a bluish tinge to it. With age the colour fades, and often changes to a yellowish green tint. These outward changes in form and colour are accompanied by modifications in the structure of the protoplasmic body itself. The chromatophore is at first perfectly homogeneous in structure, but with the fading of the colour the body passes into a granular condition, consisting of many small portions of different sizes, each having more or less of the original green tint, and all imbedded in strands of granular protoplasm. This change in structure is illustrated in the upper portion of Fig. 2, where the chromatophore is breaking up and becoming granular in texture. The process above described may be one of degeneration, and the writer has observed at different times very similar appearances in the cells of other Algae. However, it is doubtful if the structure ever entirely disappears from the trichogyne, although it may become so faint as to be noticed only by careful observations, with high powers of the microscope. It is true in general that the remains of this body are distinctly visible in most trichogynes, often with sufficient colour still left in them to make the writer feel confident of their nature

as chromatophore-derivatives. We shall have occasion to speak of the future fate of this body after we have described the process of fertilization.

We may now consider the structure and development of the antherozoids. In this species they are formed in groups at the ends of filaments towards the tips of the branches. Such a group is shown in Fig. 3, and in this case consists of three antherozoids, *a*, *b*, and *c*, in different stages of development. Perhaps the most interesting point in connexion with their development is the fact that the young antherozoids have a very distinctly outlined body in them that has the unmistakable colour of the chromatophore of vegetative cells. The appearance of this body is shown in Fig. 3 *a*. As the antherozoid matures, a change takes place in its chromatophore similar to that in the chromatophore of the trichogyne. The colour fades, the homogeneous structure becomes granular, as is indicated in Fig. 3 *b*, and the chromatophore breaks up into many smaller bodies, which finally become practically colourless, but nevertheless remain in the antherozoids as differentiated masses of protoplasm giving the granulate structure shown in Fig. 3 *c*. These bodies would be considered quite colourless by any one who had not studied their development, though they occasionally show traces of their original green tint. Each antherozoid contains a nucleus that is commonly situated in the layer of protoplasm that lies next the cell-wall (see Fig. 4).

What, now, is the real nature of the trichogyne? It starts as a process from the terminal cell of a branch, but at a very young stage a distinct nucleus is plainly recognizable in the developing structure. Thus in the young stage shown in Fig. 1, a nucleus is present in the swollen extension of the cell *c*. The extension develops into the trichogyne, the cell below (*c*) becoming the carpogonium. The nucleus in the trichogyne usually assumes a position near the middle portion of the structure, lying however in the protoplasm close to the cell-wall. But the position is not a fixed one; sometimes the nucleus is found to be at the top of the trichogyne, and some-

times near the base. It seems to the writer that the trichogyne must be considered as a true cell. There is a nucleus and a body certainly derived from a chromatophore, and which is probably functional, at least in the young trichogyne. These are the two most important structures in the vegetative cells of *Batrachospermum*, and having these, the only differences between the trichogynes and the former are those of shape, position, and mere arrangement and relative proportions of the cell-contents. The last-mentioned difference would seem to be the most important, for in the trichogyne the hyaline cytoplasm is proportionally very large, while the chromatophore is represented in its lowest terms. The trichogyne is united to the carpogonium by a wide strand of protoplasm, but this is not a great distinction, for intercellular communication is characteristic of all the cells of the frond.

Bearing in mind the nuclear and cytological conditions of the procarps, we are now ready to consider the phenomenon of fertilization. The conditions are, a nucleus in the trichogyne, a nucleus in the carpogonium, and the connexion of the two cells by a broad strand of protoplasm. When the antherozoid is applied against the trichogyne, the surfaces of the two cell-walls flatten somewhat and finally become completely united. The masses of protoplasm in the two structures are still separated by a cell-wall of appreciable thickness. The antherozoid then puts forth as a rule a very evident, although small, papilla, that apparently absorbs the cell-wall as it works its way down towards the protoplasm of the trichogyne. Finally, cytoplasmic fusion of the two cells is accomplished; but the point of union is at first very small, so small that it is impossible to determine exactly when the act takes place. The point of fusion then gradually broadens, until in some cases the opening becomes two-thirds the width of the antherozoid: the process goes on rather slowly, and, as will be shown later, the size of the opening is very variable. These points are more readily determined from fixed and stained specimens than from living material, because of the hyaline character of the protoplasm of the

latter. The antherozoids usually attach themselves near the tip of the trichogyne, and commonly at the very end. There seems to be no rule as regards the position of the nucleus in the antherozoid at the time of fertilization. It very frequently lies at the side farthest away from the point of fusion, as in Figs. 6 and 7, or it may be found very near the point of contact. The writer, after examining many specimens in the condition shown in Figs. 6 and 7, has never seen anything to indicate that the nucleus of the antherozoid or any portion of it moves rapidly into the trichogyne. The position of the nucleus of the trichogyne, as has been stated, is somewhat variable; that of the carpogonium usually lies near the base of that structure, but sometimes occupies a position above the middle portion. As it is not difficult to determine these points in this species, the writer does not see how any marked change in the nuclear conditions of the structures could have escaped his examination. There do not seem to be any violent changes in the position of the nuclei effected by the act of fusion. Gradual changes will be spoken of later. Frequently more than one antherozoid may be attached to the trichogyne, but the evidence indicates that only one fuses. The writer has examined this point with considerable care, and has never seen more than one antherozoid of a group in undoubted union with the protoplasm of the trichogyne. It is very interesting to note the manner in which all the antherozoids of a group around a trichogyne extend towards that structure so that their shape is changed from a round outline to an oval. They not only extend towards the trichogyne, but sometimes towards each other.

We will now consider the manner in which the trichogyne is separated from the carpogonium. The experiments tried upon living plants, as well as the writer's observations, seem to prove that the trichogyne never becomes separated from the carpogonium unless fertilized. The strand of protoplasm connecting the two structures is originally rather wide (see Fig. 6); and the first indication that the trichogyne is to become cut off from the carpogonium is shown by the

gradual narrowing of this strand. It is as if the masses of protoplasm in the two structures were slowly contracting; and to judge from appearances this seems to be actually the case. As the strand becomes thinner the space necessarily left between it and the cell-wall at the base of the trichogyne is filled up by a deposit of a substance exactly similar in colour and density to the cell-wall itself. This deposit is shown in Fig. 7, where the connecting strand is much thinner than in Fig. 6. Finally the thin strand is drawn apart and the ends of the two separated masses of protoplasm round themselves off, one becoming the lower end of the separated trichogyne, the other the upper end of the carpogonium. A light streak that is frequently seen running through the deposit between the carpogonium and trichogyne (see Fig. 8), may indicate the presence of the cavity in which the connecting strand originally lay. But this appearance is but transitory; and such a cavity, if it exists at all, is quickly closed up, so that the whole intervening portion of the structure between the protoplasm of the trichogyne and that of the carpogonium consists of this substance exactly similar to the cell-wall.

It will be seen that the deposit between the trichogyne and carpogonium is not exactly of the nature of a plug placed into an opening between the two, but it is an addition on the inside to the thickness of the cell-wall which is deposited as gradually as the separation of the protoplasm takes place, and follows the latter process very closely. That the separation takes place gradually is shown by the great variety of stages which one is able to find without difficulty. The writer has endeavoured to watch the operation in living material, and his attention has never been attracted to any movement of the protoplasm either during the process of fusion of the antherozoid or separation of the trichogyne from the carpogonium.

Concerning the nature of the substance deposited between the trichogyne and carpogonium, the writer can say but little. It did not give a clear cellulose-test with iodine and sulphuric

acid or with chlor-iodide of zinc; but these reagents do not give very satisfactory results with the walls of the vegetative cells. However, the general resemblance in appearance and behaviour towards certain stains has led the writer to conclude that, if not cellulose, it is at least closely related to that substance.

If it is cytoplasmic communication between the antherozoid and trichogyne which causes the separation of the latter from the carpogonium, it is interesting to note how large must be the point of fusion. In some cases, as for instance the example shown in Fig. 8, the points where the protoplasm of the antherozoids were pressed against the protoplasm of the trichogynes were so small that open communication could not be seen. While the points of fusion in the great majority of specimens were very much larger than in this case, still they are often very small, much smaller than the nuclei of the antherozoids. Fig. 9, which illustrates this point, is also interesting, because it shows the peculiar manner in which the antherozoids tend to lengthen in the direction of the trichogynes. In Fig. 10 the nuclei of the antherozoid and trichogyne both lie very close to the point of fusion. The connecting strand of protoplasm between the two structures was much smaller than the nuclei, and the trichogyne was separated from the carpogonium. The example is unusual, the only one observed, and the writer would not like to attach any particular significance to the peculiar situations of the two nuclei.

We will now consider certain gradual changes which may take place in the position of the nucleus of the antherozoid. It is true that the nucleus of the antherozoid is often found in or near the upper portion of the trichogyne, and such cases are always those in which the fusion-strand is very broad. Fig. 11 illustrates this point, the nucleus lying directly in the opening between the antherozoid and trichogyne. In the example shown in Fig. 12, the nucleus of the antherozoid had passed into the upper portion of the trichogyne, and there was a peculiar arrangement of the cytoplasm before

and behind the nucleus which indicated that the position of the cell-contents had been disturbed by the downward movement. The attention of the reader is called to the fact that the nuclei of the trichogynes in both cases occupy their usual positions in the middle portion of those structures, and that the two trichogynes are entirely separated from the carpogonia. In the specimen illustrated by Fig. 11, the fertile filaments had begun to develop, showing that the carpogonium had been fertilized.

There appears to be no tendency on the part of the nuclei of the antherozoid and trichogyne to approach each other. It would be very unsafe to conclude that the examples in which they lay very close together had any great significance, because the position of the nucleus of the trichogyne is so very variable.

In the majority of cases it is quite evident that the nuclei of the antherozoid and trichogyne remain entirely separate. It is true that one sometimes finds specimens, as Fig. 17, in which there is one large nucleus that might be considered as a fusion-nucleus; but there have been other structures in such cells that have led the writer to believe that such was not the case, and these will be described later.

The carpogonium, soon after its separation from the trichogyne, generally gives rise to several branches of three or four cells each, that grow downward and become pressed against the cell below. A great many short filaments arise from the cells of these primary branches, and they in turn fork and finally terminate in the spores. All the fertile filaments may be traced back to the carpogonium, although they frequently appear to come from the cell situated below that structure. It may be well to emphasize the fact that after the trichogyne is separated from the carpogonium, all communication between the two structures is for ever ended. The communication is never opened again, either by the absorption of the deposit between the two cells or by outgrowths from the trichogyne. The writer mentions this point to guard against any possible

attempt to account for a transfer of the nucleus of the antherozoid to the carpogonium by ooblastema-filaments put out from the trichogyne.

It is characteristic of the trichogynes of *Batrachospermum* that they remain a long time in good condition, only disappearing with the gradual disruption and decay of the whole cystocarp. But several changes often take place in the character of the cell-contents. The nucleus usually increases in size, and often a very interesting process of fragmentation takes place. This latter phenomenon may occur in the antherozoid, in the trichogyne, or in both structures at the same time. It consists of a gradual breaking up of the nucleus into two or more smaller masses, which still take the dark blue stain with haematoxylin. Fig. 13 illustrates a simple case of fragmentation in both antherozoid and trichogyne. In Fig. 14, two large portions of the nucleus in the trichogyne lie one at the base and the other near the middle of that structure. The nucleus of the antherozoid is very granular, and perhaps is about to split up into a great many small fragments of the character shown in the antherozoid of Fig. 15. Fig. 16 illustrates an interesting case in which there were two well-defined nuclei in the antherozoid, and the nucleus of the trichogyne lay at the top of that cell. The phenomenon of fragmentation often gives rise to appearances which might be incorrectly interpreted if one were not familiar with the process. In Fig. 17 there is shown a large prominent nucleus in the upper portion of the trichogyne that might be considered as a fusion-nucleus; but the protoplasm in the central portion of the trichogyne was very dark, and gave indications of organization, and the question might well be asked whether it was not the remains of the nucleus of the trichogyne. Again, the cell-contents of the antherozoid may be almost hyaline but for a few scattered dark granules, and there may be one large nucleus in the trichogyne; but it is quite possible that fragments of a divided nucleus as small as those shown in Fig. 15 might readily be quite lost when scattered in the cytoplasm. If the nucleus of the antherozoid

ever fuses with that of the trichogyne, indications of the process ought to appear in the younger specimens, and it is very unsafe to draw conclusions from old examples. The nuclei in these structures often do not undergo any material change: Fig. 18 illustrates such a case, the example being part of a crushed-out specimen of an adult cystocarp with fertile filaments and ripe spores (lettered *s*).

In living specimens of old trichogynes certain portions of the cell-contents frequently appear to have a greenish tinge; and not only the trichogynes but also the antherozoids contain these peculiarly coloured portions of the protoplasm. The writer has observed some instances where the colour was so distinctly green and the outline of the body so well defined, that it seemed reasonable to suppose it to be really of the nature of a chromatophore. One sometimes finds antherozoids which have not fused with the trichogyne that are quite green in colour. Fig. 5 shows the arrangement of this chromatophore-like body in one case, but there is no regularity in its form or position. The body takes a slightly deeper stain with haematoxylin than the rest of the cytoplasm, perhaps because of its granular character, and the outline is sometimes well shown in preserved and stained specimens (see Figs. 8, 9, 10, and 11). Remembering how long the trichogynes and the antherozoids remain intact, it does not seem unreasonable to suppose that the trace of colour may be true chlorophyll with some functional value. From the appearance of specimens which the writer has observed, it seems to him that the colour deepens in tint after fertilization, and that the chromatophores become larger.

The writer realizes that the process which he has called fertilization lacks the characteristics of that phenomenon as considered by biologists, in that there is no fusion of sexual nuclei. Nevertheless, it is the act of cytoplasmic fusion with the antherozoid that gives the stimulus necessary for the development of the cystocarp, and as such the term is perhaps not misapplied.

BATRACHOSPERMUM COERULESCENS, *Sirdt.*

This plant, which agrees more closely with *B. coerulescens*, as Sirodot[1] has described it, than with any other species, was found in its best condition in the late fall, but it grows all through the winter and spring. The types of trichogyne and cystocarp are of such an opposite character from *B. moniliforme* that a careful comparison of the two species as regards the process of fertilization is very desirable.

We shall take up the subject in much the same order as in *B. moniliforme*, so that the comparison may be easily followed.

The procarpic branch is very short, lying near the axis of the main branches, and bears a very narrow long trichogyne that arises from the terminal cell (the carpogonium). There are curious short filaments attached to the cells of the procarpic branch below the carpogonium, but they are of no great significance.

The mature trichogyne contains a well-defined nucleus, as does also the carpogonium, but the most interesting feature about the contents of the trichogyne is the particularly prominent chromatophore-derivative. In very young trichogynes, when the process from the carpogonium is quite small (see Fig. 19), a homogeneous body of irregular outline may be clearly seen closely pressed against the cell-wall and extending usually to the very top of the cell. Its colour is distinctly green, and its outline may be followed without difficulty, as it runs into the carpogonium and there becomes the chromatophore of that structure. As the process, which represents the early stage of a trichogyne, gradually lengthens and swells into the shape more nearly like the mature structure, the chromatophore, which lay at first closely pressed against the cell-wall, is commonly left behind in the lower portion of the structure (see Fig. 20). The shape finally assumed by the chromatophore-derivative is very variable, and frequently lobes of the structure become detached (Figs.

[1] Sirodot, Les Batrachospermes, Paris, 1884.

20 and 21). Eventually the chromatophore of the trichogyne becomes separated from that of the carpogonium, as in Fig. 21, an example of a mature trichogyne in which this body is particularly well developed. As the trichogyne grows old, the colour of the chromatophore gradually changes from the peculiar somewhat bluish-green tint to a green with a trace of yellow in it, becoming at the same time somewhat fainter in colour. With the change in tint comes the same difference in the character of the protoplasm which we have considered in the case of *B. moniliforme*, namely, a change from a homogeneous appearance to a granular structure with a less definite outline.

The writer found several instances of an abnormal development of the trichogyne that are worth noticing. The terminal cell of a procarpic branch had given rise to a process, but the latter, instead of developing directly into the trichogyne, had grown out and divided into two cells, thus continuing the growth of the branch (see Fig. 22). This exhibition of vegetative character in the young trichogyne is exceptional and interesting, but quite in keeping with its structure as a nucleated cell.

The phenomenon of fertilization may be studied very advantageously here, because the antherozoid sends out a very distinct process. After the act of protoplasmic fusion, the long delicate strand of cytoplasm between the trichogyne and carpogonium (see Fig. 23) becomes thinner, and finally breaks. The separated ends contract, but do not round themselves off quickly. Little extensions from the upper portion of the carpogonium and lower portion of the trichogyne are frequently found when these structures must have been long separated from each other. Between the separated portions of protoplasm is deposited a substance apparently of the same nature as the cell-wall. The nuclei of this species are very small, but they stain very deeply indeed. That of the trichogyne usually lies near the middle portion of the cell, but occasionally at the tip (see Fig. 25). The nucleus of the antherozoid is very irregular in its situation, and

frequently occupies a position farthest away from the point of fusion (see Figs. 24, 25, and 28). It will be noticed from the figures that the point of fusion is at first very small, and it sometimes remains so for a long time, although as a rule it gradually widens. However, the process of widening takes place particularly slowly in this species, and the tendency on the part of the nucleus of the antherozoid to pass into the trichogyne is much less noticeable than in *B. moniliforme*. In fact, cases in which the nucleus of the antherozoid had left that organ, as in Fig. 26, were rather infrequent. We must make special mention of one very interesting specimen (Fig. 27). Here there was no nucleus in the trichogyne proper; but just inside the antherozoid, near the point where the two structures were united, was one nucleus, and a second lay in the antherozoid at the side farthest away from the point of fusion. Apparently the nucleus of the trichogyne originally lay near the tip of that structure, as in Fig. 25, and when fusion took place the movement of the cytoplasm was into the antherozoid, and the surge carried the nucleus of the trichogyne with it. Certain appearances of the protoplasm around the nucleus of the trichogyne tended to support this explanation.

Although more than one antherozoid may be frequently found clinging to the trichogyne, as in Fig. 28, only one ever fuses, and this plant was particularly favourable for the study of this point. Specimens were occasionally observed in which antherozoids were attached to the base of the trichogyne (Fig. 29), but there never appeared to be protoplasmic fusion under such conditions.

The interesting phenomenon of nuclear fragmentation is very characteristic of this species. The process does not, as a rule, begin until the trichogyne is cut off from the carpogonium as an independent cell. However, in the example shown in Fig. 23, a specimen in which the trichogyne was still united to the carpogonium, the nucleus of the trichogyne was very large and irregularly elliptical in its outline, as though it were about to divide. Two fragments of the

nucleus of the trichogyne are shown near together in Fig. 28, while a similar condition in the antherozoid is indicated in Fig. 30; and in the specimen from which Fig. 31 was drawn, fragmentation had taken place in both structures at the same time. The nuclear fragments may finally be quite widely scattered in the cytoplasm (Fig. 29 and 32, *t*), and the process of division is sometimes carried on until several derivatives of the primary nucleus exist in the cell.

The essentials of the structure and development of the cystocarp are precisely the same as in *B. moniliforme*, although there are minor differences in the length and arrangement of the fertile filaments. The appearance of a crushed-out developing cystocarp is shown in Fig. 32, where *t* = trichogyne, *c* = carpogonium, and *s* = sterile filaments from the cell below the carpogonium.

Batrachospermum coerulescens therefore agrees with *B. moniliforme* in all the important characteristics of the phenomenon of fertilization. Certain structures of the trichogyne and antherozoids are illustrated much better, as for example the presence of the chromatophore-derivative in the trichogyne before and after the fertilization of the carpogonium; in fact, it was here that the attention of the writer was first called to this curious cytological condition.

Batrachospermum Boryanum, *Sirdt.*

The trichogyne of *B. Boryanum* is very variable in size and shape, ranging from an almost globular structure to a lengthened form in which the end is sometimes very much prolonged. The general type of procarp is, however, somewhat similar to *B. moniliforme*. The cells are smaller than in the two species previously described, and consequently it is a less satisfactory form to study; but the writer made as careful an examination of the cytological conditions here as in the other species.

There is a nucleus in the trichogyne as well as in the carpogonium; and in the former there is also a more or

less clearly differentiated portion of the protoplasm, having a greenish tint: in young specimens this body may be readily traced back into the carpogonium, where it joins the chromatophore of that structure. The antherozoids of this plant are unusually large, and are somewhat peculiar in that the protoplasm is frequently entirely confined to a rather thin layer next the cell-wall, the central portion being occupied by a large cavity or vacuole. Half-developed antherozoids contain a very distinct chromatophore. The colour and structure of this body gradually change as the antherozoid matures, in a manner precisely similar to the antherozoids of *B. moniliforme*; i. e. the green tint becomes fainter and the structure granular and less definite in outline.

The separation of the contents of the trichogyne from the carpogonium takes place in exactly the same manner as in the two other plants. The area of the point of fusion between the antherozoid and trichogyne may be very small to accomplish the fertilization of the carpogonium (see Figs. 35, 36, and 37). The position of the nucleus in the trichogyne is apparently not materially affected by the process of fusion. The nucleus of the antherozoid is very irregular in its situation, and one finds many instances where its position is remote from the point of fusion, as in Fig. 33; but it is often very near this point (Fig. 34). The nucleus of the antherozoid frequently passes into the trichogyne, but no indications were observed that it ever moved far down into that structure. Fig. 33 shows a particularly interesting case in which, if one may judge by the position of the granular protoplasm in the upper portion of the trichogyne, the movement of the cytoplasm was into the antherozoid rather than from it. Fig. 35 illustrates a type of trichogyne with a prolonged upper portion in which the nucleus was situated: but one also finds examples in which the nucleus lies below the middle portion of the cell.

Nuclear fragmentation takes place in the cells of the antherozoid and trichogyne as these structures grow old. Some of the figures illustrate this phenomenon: thus Figs. 35 and 36 show fragmentation in the antherozoid, and

Fig. 37 is an example in which it has begun in both structures. In the specimen shown in Fig. 38 there was no prominent nucleus in the fused antherozoid, whilst there were two well-marked nuclei in the lower portion of the trichogyne; but the presence of a number of darkly staining bodies in the antherozoid suggests the possibility of extensive division of its nucleus, while the basal position of the two nuclei in the trichogyne seems to be good evidence that they were once portions of the same structure. In this species also the old trichogynes and antherozoids attached to developing cystocarps frequently contained differentiated portions of the protoplasm that had a faint but definite greenish colour. The writer is compelled to consider them, at least morphologically, as true chromatophores, for they appear to be the remains of the chromatophores contained in the young trichogynes and antherozoids.

Summary and Conclusions.

The writer has reserved this portion of the paper for a consideration of the bearing of the observation just described upon the accepted idea of the structure of the procarps and of the process of fertilization in the genus. The problems involved in a satisfactory explanation of the process of fertilization are of great interest but very puzzling: we cannot, indeed, expect a solution of the questions until many more species have been studied in this genus and related forms. The number of species that the writer has examined is of course small, and they all come from the same locality. However, there is this to be said to their advantage, that each species represents a different sub-division of the genus as it has been considered by Sirodot. The sub-division **Moniliformes** is represented by *B. moniliforme*, that of the **Helminthoides** by *B. Boryanum*, and the **Verts** by *B. coerulescens*. The two extremes of the genus are thus represented: for *B. moniliforme* and *B. coerulescens* are types in which the procarps present the greatest differences in the size and form of the cells.

The following *résumé* of the principal points of the paper had best precede the consideration of the literature, when each topic may be discussed in turn.

I. *The trichogyne* is a cell, possessing a well-defined nucleus, and when young, a body that must be considered, at least morphologically, as a chromatophore. The chromatophore becomes less clear in outline and fainter in colour as the trichogyne develops, but traces of it may usually be found in the mature structure.

II. *The carpogonium* is the cell situated directly below the trichogyne and connected with it by a strand of protoplasm. It contains a centrally-placed nucleus.

III. The *antherozoids* in an early stage of their development contain a body certainly derived from the chromatophore of the vegetative cells, and having a distinct green colour. As an antherozoid matures, this chromatophore-derivative changes greatly in appearance, its structure becoming granular and the colour fading until the antherozoid is practically colourless. The nucleus is usually situated in the layer of protoplasm near the cell-wall, the central portion as a rule containing a large vacuole.

IV. *The fertilization* of the procarp is accomplished when the trichogyne becomes separated from the carpogonium. The process of separation consists of a gradual drawing apart of the cell-contents of the two structures until the connecting-strand becomes so thin that it breaks. The cavity left by the separation of the two masses of protoplasm is filled in by a deposit of a substance similar in character to the cell-wall. The exciting cause of the process of fertilization is the *cytoplasmic* fusion of one antherozoid with the contents of the trichogyne. The evidence to support this statement is of two kinds. First, as shown by isolation-experiments on living plants, the fusion of an antherozoid is necessary for the further development of the carpogonium. Second, it appears that the process of separation may take place when the point of union between antherozoid and trichogyne is very small,

and that the nucleus of the antherozoid need not even enter the trichogyne to accomplish this act of fertilization.

V. *The nucleus of the antherozoid* has no fixed position in that structure at the time of fertilization, and frequently is situated at the side farthest removed from the point of fusion. The nucleus may never leave the antherozoid, but if the opening is large it may pass down into the upper portion of the trichogyne. Although it frequently enters the trichogyne, there is no particular tendency on its part to move down that structure, and the position of the cell-contents of the latter does not appear to be materially affected by its entrance.

VI. *A process of fragmentation* of the nuclei of both antherozoid and trichogyne is very apt to begin soon after the fertilization of the carpogonium.

VII. *The cystocarp* consists of many fertile filaments, all of which may be traced back to the carpogonium. When the communication between the trichogyne and carpogonium is severed, all protoplasmic union is for ever ended between the two structures.

VIII. *In the old trichogynes and antherozoids* one may find differentiated portions of the protoplasm having a faint green tint. These are probably the remains of the chromatophore-derivatives of the young antherozoids and trichogynes. Having a green colour, it is possible that they are functional, and that the long life of the trichogynes may be attributed to their presence.

The idea of the trichogyne of *Batrachospermum* being a distinct cell with its own nucleus and also a chromatophore-derivative is quite new. From the study of certain other genera of Florideae, the writers upon the subject, Bornet and Thuret [1], Janczewski [2], and Schmitz [3], have considered the

[1] Bornet et Thuret, Recherches sur la Fécondation des Floridées. Ann. d. Sci. Nat., Bot., 5e série, t. vii, 1867.

[2] Janczewski, Notes sur le Développement du Cystocarpe dans les Floridées. Mém. d. la Soc. Nat. d. Sci. Natur. d. Chérbourg, t. xx, 1877.

[3] Schmitz, Untersuchungen über die Befruchtung der Florideen, Sitzgb. d. k. Akad. d. Wiss. z. Berlin, 1883.

trichogyne to be always a prolongation of a female cell, specialized to receive the antherozoid. This conception is well illustrated in the case of *Nemalion*, for there the trichogyne is a mere extension of the cytoplasm from the carpogonium, and of course has no independence of character as a cell. The female nucleus is supposed by all these writers to lie in the swollen portion of the cell below the trichogyne, which is now pretty generally spoken of as the carpogonium. The general tendency of thought is then to consider the carpogonium as homologous with the oogonium of certain chlorophyllaceous Algae, and the resemblance between the typical carpogonium with its trichogyne and the peculiar oogonium of members of the Coleochaetaceae has often been noticed. However well such a conception may apply in many genera of the Florideae, the writer does not think that *Batrachospermum* can be considered in such a light. Its trichogyne, with a nucleus and a body morphologically a chromatophore, is not a mere prolongation of a cell.

It may be mentioned that Schmitz [1] states, with an accompanying figure of *B. moniliforme*, that he has observed fragments of a substance, staining as chromatin reacts, in the trichogyne after it has been cut off from the carpogonium. He regarded these nuclear fragments as being evidence of a reduction-process, whereby some of the chromatin of the female cell is discarded. It is of interest to confirm his observations upon *Batrachospermum*, although the writer cannot put the same interpretation upon the facts; for the nuclear fragments are probably derived from the nucleus of the trichogyne.

The writer, while using the term fertilization throughout this paper, has realized that the process which he has described would not be called a sexual process by most biologists. From the observations here recorded it does not appear that the nucleus of the antherozoid, or any portion of it, ever reaches the carpogonium. Even when the nucleus left the

[1] Schmitz, l. c., p. 13.

antherozoid, nothing more remarkable ever happened than its passage into the upper portion of the trichogyne. The cytoplasmic fusion of an antherozoid with the trichogyne cannot be considered as a sexual process in the usually accepted meaning of the expression, for the essential characteristic of the process is considered to be the intimate union of the substances of sexual nuclei. However, the isolation-experiments and all observations seem to prove that the union of an antherozoid with the trichogyne is necessary for all farther development of the procarp, and also that it is cytoplasmic fusion between the two which is the exciting cause of the separation of the trichogyne from the carpogonium. It is hard to suggest what other term than fertilization can be used to describe this act, because all the subsequent changes are apparently the direct sequence of the influence of an antherozoid upon the trichogyne.

What then can be the meaning of these curious conditions? There seem to the writer only two possibilities open:— (1) Perhaps the conditions of sexual reproduction among the lower plants are such that the fusion of the nuclei of the two sexual cells is not absolutely necessary: possibly a stimulus of some sort, the result of cytoplasmic fusion, is all that is required. Such a stimulus could readily be conducted from one cell to the other by the wide strand of protoplasm between the trichogyne and carpogonium. (2) The second possibility is that the present condition is a much modified form of what was once a true sexual process, the plant having lost the most important feature of the process (the union of sexual nuclei), but the stimulus of cytoplasmic fusion being still required: that is, the plant is tending towards a condition of apogamy. The present trichogyne may then be a modification of some ancestral type of structure when the procarp resembled that of *Nemalion* at the present time.

As we know very little about the fate of nuclei in the sexual cells of the lower Cryptogams during the process of fertilization, it is quite impossible to draw any analogies.

From the present trend of theories of heredity one would suppose the first suggested possibility very improbable. But the second condition would be also very remarkable indeed. It will perhaps be safest to defer judgement until we have more detailed information about the process of fertilization in allied forms of the Florideae and other Algae.

UNIVERSITY OF CHICAGO,
October 1, 1895.

EXPLANATION OF FIGURES IN PLATES VI AND VII.

Illustrating Mr. Davis' paper on *Batrachospermum*.

All figures sketched with the Camera lucida.

PLATE VI.

Batrachospermum moniliforme, Roth.

Figs. 6–18. Drawn from specimens fixed in absolute alcohol and stained with Mayer's acid haemalum, glycerine preparations. × 2400.

Fig. 1. Procarpic branch with very young trichogyne developing from carpogonium-cell (*c*), stained with Mayer's acid haemalum. × 2400.

Fig. 2. A trichogyne drawn from life, showing distribution of chromatophore-derivative. × 1600.

Fig. 3. A group of antherozoids (lettered *a*, *b*, and *c*) in different stages of development, illustrating the gradual change that takes place in the chromatophore as the structure matures. Drawn from life. × 2400.

Fig. 4. A group of developing antherozoids, stained with Mayer's acid haemalum. × 1600.

Fig. 5. An old trichogyne, showing the chromatophore-derivative as it appears in this structure and in the two antherozoids at the right and left. × 2400.

Fig. 6. Trichogyne united to carpogonium; nucleus of antherozoid at further side of that structure.

Fig. 7. Antherozoid at the left fused with the trichogyne; that at right merely applied against the cell-wall; cell-contents of trichogyne about to separate from the carpogonium.

Fig. 8. Illustrates an instance where the point of fusion is very small.

Fig. 9. Fertile filaments beginning to develop from the carpogonium.

Fig. 10. Shows peculiar and very exceptional position of the nuclei of the trichogyne and antherozoid.

Fig. 11. Nucleus of antherozoid near point of fusion.

Fig. 12. Nucleus of antherozoid having passed into the upper portion of the trichogyne.

Fig. 13. Illustrates fragmentation of nucleus in trichogyne and antherozoid.

Fig. 14. Nucleus of antherozoid breaking up into many small fragments.

Fig. 15. Nucleus of antherozoid in many small fragments, probably derived from such a condition as that shown in Fig. 14.

Fig. 16. Two nuclei in fused antherozoid.

Fig. 17. Shows manner in which fertile filaments grow downward from the carpogonium (*c*); prominent nucleus from antherozoid in upper portion of the trichogyne.

Fig. 18. Portion of a mature cystocarp showing trichogyne (*t*) and antherozoid; each containing its original nucleus; *s*, developed spores.

Batrachospermum coerulescens, Sirdt.

Figs. 19–23 drawn from living specimens.

Fig. 19. Carpogonium with very young stage of trichogyne; illustrates distribution of chromatophore in that structure. × 2400.

Fig. 20. Half-developed trichogyne with chromatophore extending from the carpogonium. × 1600.

PLATE VII.

Figs. 23–32 drawn from glycerine preparations of specimens fixed with absolute alcohol and stained with Mayer's acid haemalum, magnification 1600 diameters.

Fig. 21. Mature trichogyne with chromatophore-derivative. × 1100.

Fig. 22. A peculiar instance where the trichogyne-cell has continued to grow forward and has divided into two cells. × 1600.

Fig. 23. Trichogyne still connected with carpogonium.

Fig. 24. Nucleus of antherozoid lies in a position farthest removed from point of fusion.

Fig. 25. Nucleus of trichogyne lying very near the top of that structure.

Fig. 26. Nucleus of antherozoid having passed into the trichogyne.

Fig. 27. Nucleus of trichogyne having passed into the antherozoid, probably derived from a condition of affairs such as is shown in Fig. 25.

Fig. 28. Illustrates fragmentation of nucleus of the trichogyne.

Fig. 29. A condition similar to Fig. 28, except that the nuclear fragments in the trichogyne are widely separated; antherozoid near base of trichogyne.

Fig. 30. Shows fragmentation of nucleus of the antherozoid.

Fig. 31. Fragmentation in both trichogyne and antherozoid.

Fig. 32. A half-developed cystocarp; *t*, trichogyne with nuclear fragments; *c*, carpogonium; *s*, short sterile branches.

Batrachospermum Boryanum, Sirdt.

All figures drawn from specimens fixed in absolute alcohol, glycerine preparations, and stained with Mayer's acid haemalum ; × 2400 diameters.

Fig. 33. A very short type of trichogyne, apparently the surge of the protoplasm during the process of fusion was into the antherozoid.

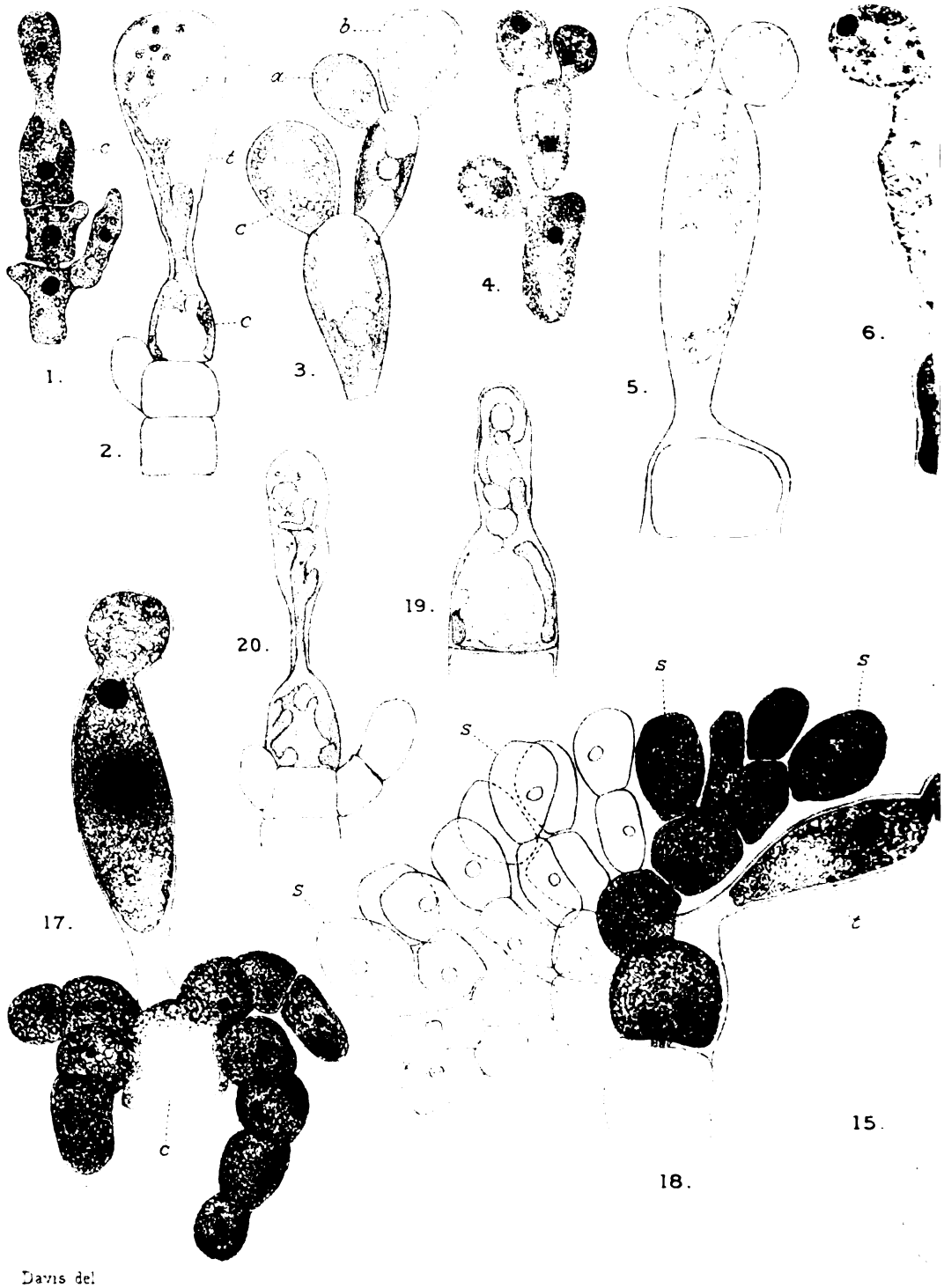
Fig. 34. Nucleus of antherozoid near the point of fusion.

Fig. 35. An elongated type of trichogyne with nucleus near the tip ; illustrating fragmentation of nucleus of the antherozoid.

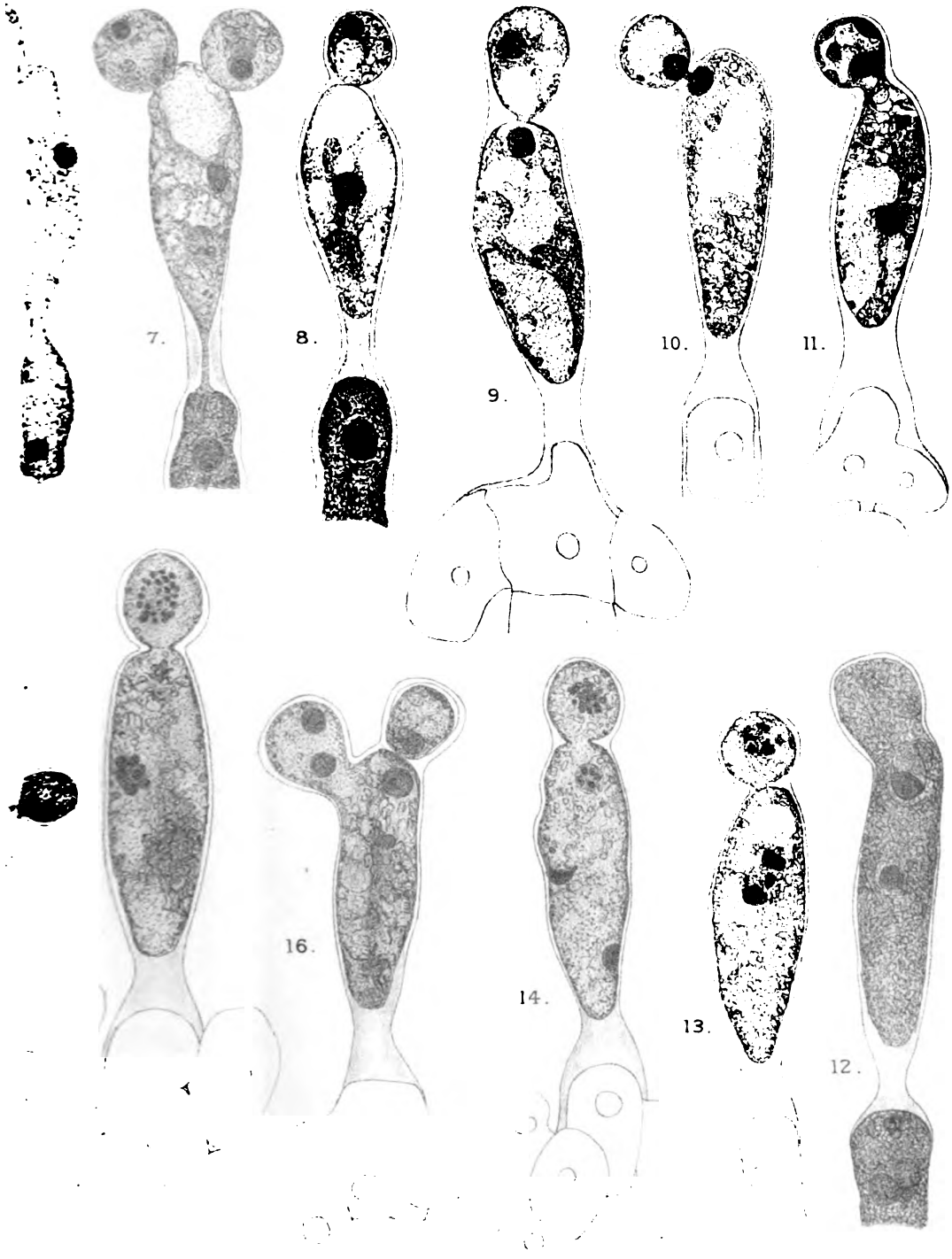
Fig. 36. Fragmentation of nucleus of the antherozoid.

Fig. 37. Nuclear fragmentation in both antherozoid and trichogyne.

Fig. 38. Two large fragments of nuclei at the base of the trichogyne; nucleus of antherozoid probably very much divided and scattered in the cytoplasm.



DAVIS. — BATRACHOSPERMUM.



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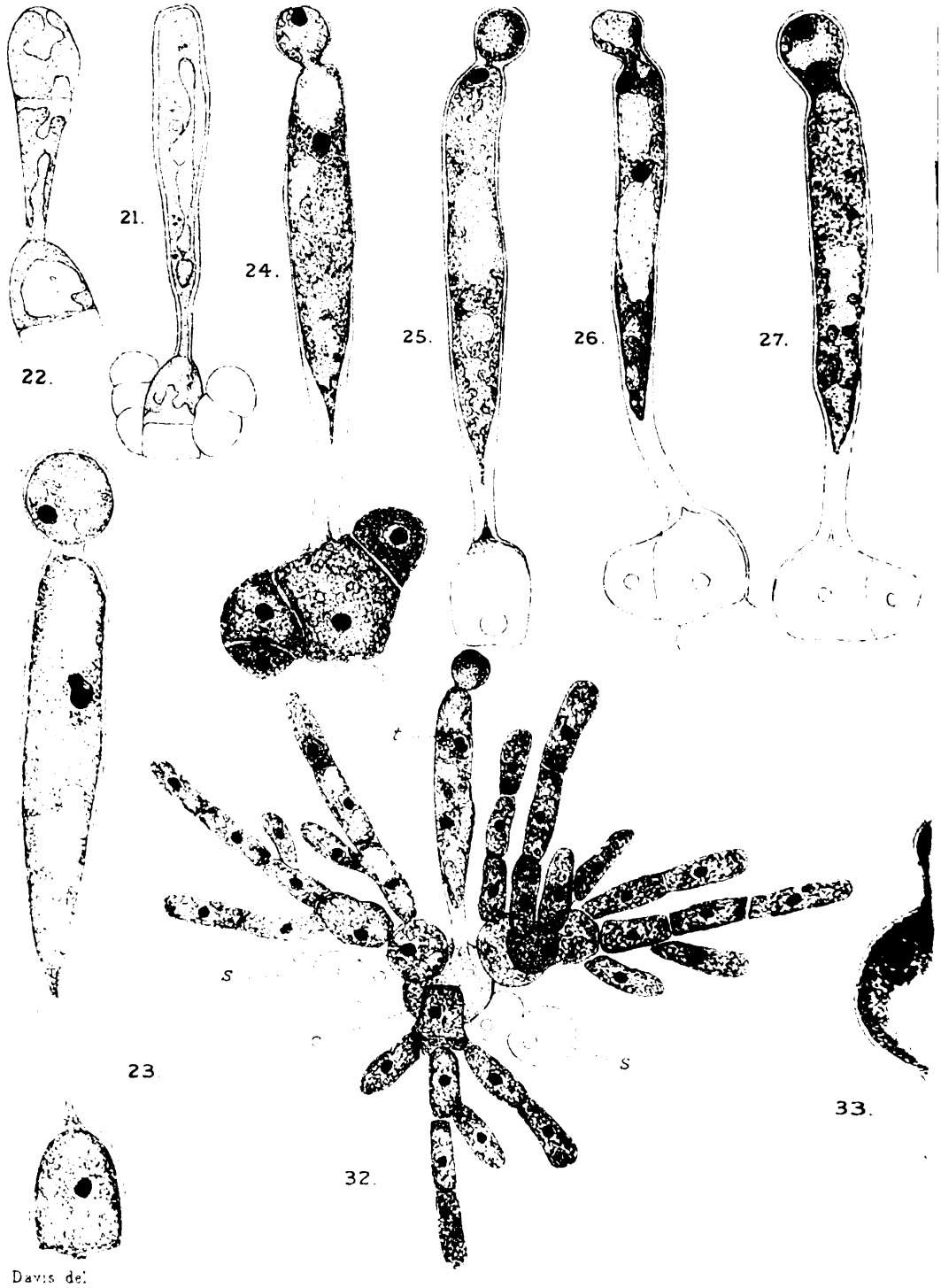

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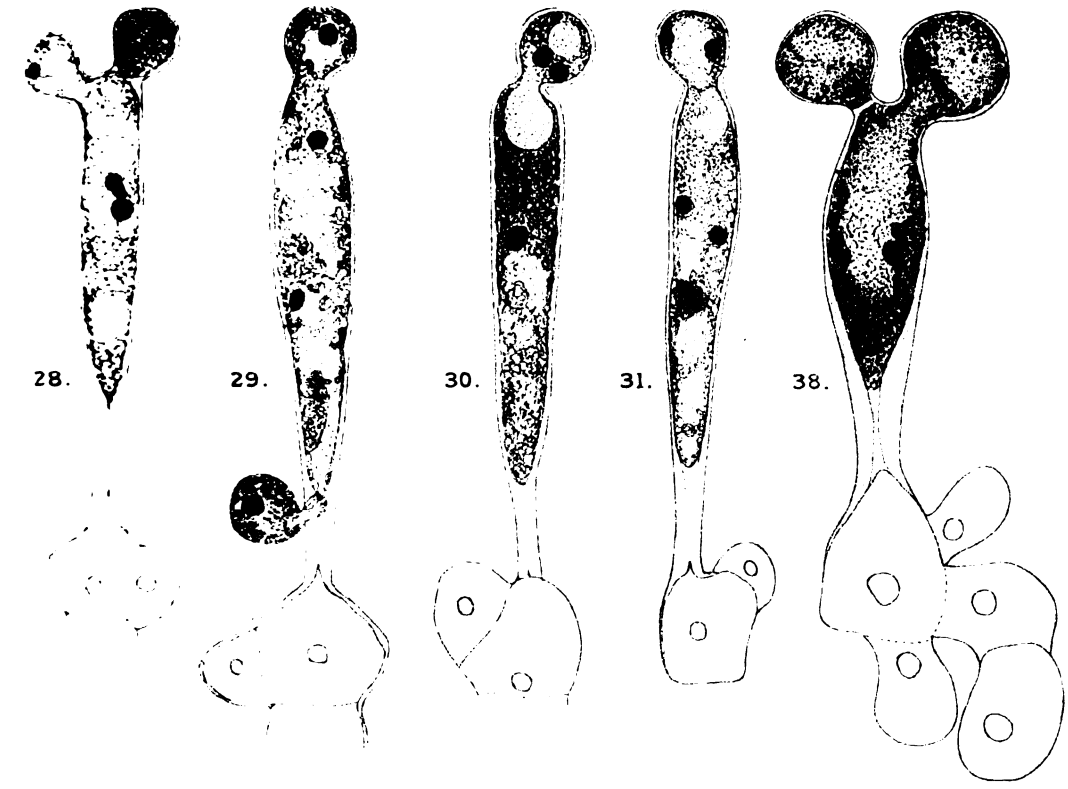

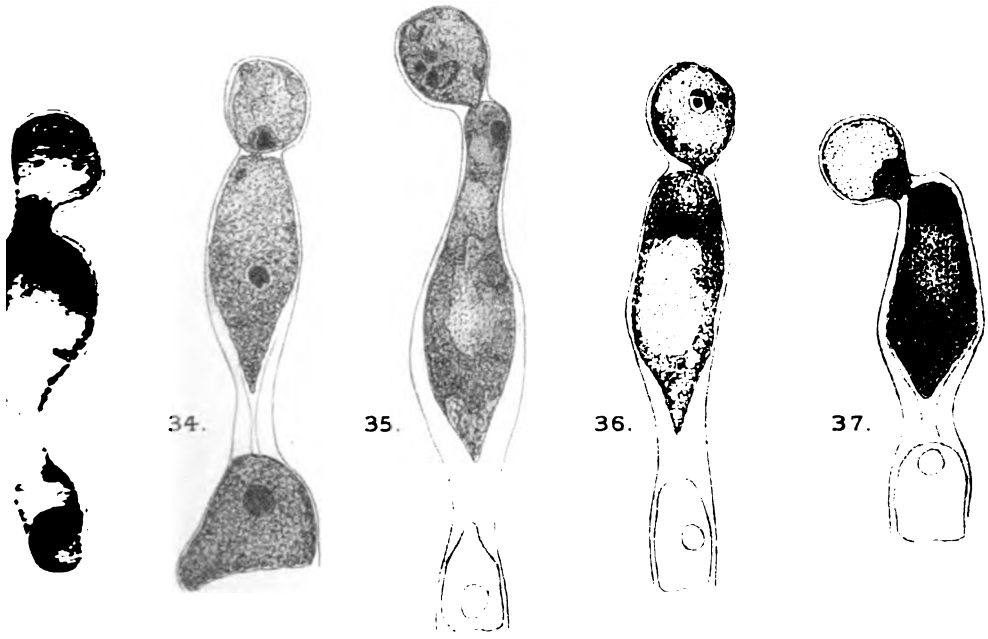

University Press, Oxford.

Contributions towards a Knowledge of the Anatomy of the genus Selaginella, Spr.

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With Plate VIII.

PART II. THE LIGULE.

THE present paper is the third of a series[1] in which I desire to record observations on the minute anatomy of the genus *Selaginella*, and deals more especially with the structure and development of the ligule.

Accounts of the structure and development of the ligule in *Selaginella* are few and far between. Russow[2], in discussing the structure of the leaf in this genus, refers to the ligule merely as a means of distinguishing one leaf-face from the other,—that next the stem being 'ligular,' that away from the stem 'aligular.'

Treub[3] says: 'Je n'ai pas étudié en detail la formation de la ligule,' and both Dangeard[4] and Erikson[5] ignore its

[1] Annals of Botany, Vol. vii. No. xxvii, 1893, and Vol. viii. No. xxx, 1894.

[2] Vergleichende Untersuchungen über Leitbündel-Kryptogamen. Mém. l'Acad. Imper. St. Pétersb. xix. 1872.

[3] Les organes de la végétation du *Selaginella Martensii*, Spr. Leide, 1877.

[4] Essai sur l'anatomie des Cryptogames vasculaires. Le Botaniste, Vol. i, 1889.

[5] Bidrag till Kännedomen om Lycopodinebladens Anatomi. Arbet. fran Lunds Bot. Instit., 1892.

[Annals of Botany, Vol. X. No. XXXVII. March, 1896.]

existence altogether. Hofmeister[1] gives by far the fullest account of the development of the ligule. His observations were made chiefly on *S. denticulata*, Spr., and *S. Galeottei*, Spr. He describes it as arising from a double row of cells at the base of the young leaf, just in the angle between the leaf and the stem. These cells remain larger than the neighbouring cells, and have their free walls curved outwards. By alternate oblique segmentation of the cell lying further from the stem, there comes to be formed a ridge of cells with an apical merismatic region lying at right angles to the long axis of the leaf and close to its base. Longitudinal and transverse divisions follow, so that the body of the ligule becomes several layers thick. Terminally the ligule is only one layer of cells in thickness, and is fringed with unicellular papillae. The cells by which the ligule is sunk in and attached to the leaf-base remain always as two parallel rows. Hofmeister describes the cells of the ligule as containing a granular colourless slime, and as being destitute of chlorophyll. He also draws attention to the very transitory vitality of the ligule, and to the fact that the development of the ligule is concluded long before that of the leaf to which it belongs. These two observations seem to be of great importance as bearing on the probable homologies and functions of the ligule: to this, however, I shall refer later on.

Pfeffer[2], in tracing the development of the embryo in *S. Martensii*, makes a brief reference to the mode of origin of the ligule. He describes it as arising from a single row of four to six cells, not from a double layer, as described by Hofmeister; but his account of the further development agrees substantially with that of Hofmeister.

McNab[3] draws attention to the expansion of the leaf-trace bundle beneath the base of the ligule, and suggests that the ligule is an organ of absorption.

[1] Vergleichende Untersuchungen der Entwicklung höherer Kryptogamen. Leipzig, 1851.

[2] Die Entwicklung des Keimes der Gattung *Selaginella*. Hanstein's Bot. Abhandl. 1871.

[3] The Stomata and Ligules of *Selaginella*. Brit. Assoc. Rep. 1887.

Farmer[1] contrasts *Selaginella* with *Isoetes* in regard to the structure and development of the ligule, and points out that in the former the ligule is multicellular in origin, whilst in the latter it is unicellular. He further remarks that 'the mature structure of the ligule is not nearly so complete as in *Isoetes*, especially as regards its insertion.' My observations on the ligule of *Selaginella* scarcely bear out this view. The ligule, Farmer states, is developed much later in *Selaginella* than in *Isoetes*.

Bower[2] gives no description of the development of the ligule, but in his figures illustrating the development of the sporangium he gives certain data worthy of comment: his Fig. 79 (Pl. 47) shows a primary stage in the development of the ligule of *S. Martensii*, where two shaded cells (in longitudinal section) indicate its point of origin, whilst Figs. 84, 85, 86, 87, and 89 illustrate stages in the development of the ligule of *S. spinosa*, to which I shall have occasion to refer later. Professor Bower has been so kind as to place his preparations at my disposal for comparison with my own, and I may say that they fully bear out his figures.

Campbell[3], in his recent work on Mosses and Ferns, gives a brief reference to the ligule in *S. Kraussiana*, and speaks of it as 'much constricted at the point where it joins the leaf.' His figure (Fig. 261), moreover, shows the sheathing-cells of the glossopodium not continuous with the epidermal-cells, but quite outside them.

It will be seen from this short summary that our knowledge of the variations in the adult form of the ligule and its comparative development in different species is, to say the least, meagre; whilst we have conflicting accounts of its mode of development in the works of Pfeffer and of Hofmeister, the former describing it as arising from one row of cells in *S. Martensii* ['wie bei diesen ensteht am Grunde der Innenseite der Kotyledonen eine Ligula, an deren Bildung ich immer

[1] On *Isoetes lacustris*, L. Annals of Botany, Vol. v, 1890.
[2] Studies on the Morphology of spore-producing Members. Phil. Trans. 1894.
[3] The Structure and Development of Mosses and Ferns. Macmillan & Co. 1895.

nur eine einfache . . . . Reihe von vier bis sechs Zellen Theil nehmen sah'], whilst the latter believes it to arise in *S. denticulata* and *S. Galeottei* from two rows of cells. It may be noticed at this point that Bower's figures for *S. Martensii* show two rows of cells in longitudinal section, not one as described and figured by Pfeffer.

In the present paper I have endeavoured (*a*) to give a general and comparative account of the form and adult structure of the ligule, employing for that purpose alcoholic material of over fifty species, (*b*) to describe the chief stages in the development of the ligule in a few selected types, and (*c*) to hazard such suggestions as to the homologies and functions of the ligule in the genus as seem to me to be borne out by the data I have endeavoured to collect.

A. Adult Structure of the Ligule.

The ligule, in the majority of the species which I have examined, arises just at the junction of the stem and leaf-base, but distinctly from the leaf. In forms like *S. oregana* (Fig. 19), *S. rupestris*[1], &c., however, the ligule is seated in a deep pit in the leaf-base, and with a well-marked swollen region intervening between it and the stem, so as to suggest in some measure the foveola and ligule of *Isoetes*, and the very deep-seated 'ligules' of allied fossil forms.

Generally speaking, the ligule varies in outline from a short, somewhat rectangular plate, whose free distal margin may be more or less fringed with unicellular papillae, as in *S. Douglasii*, *S. stenophylla*, *S. suberosa*, *S. molliceps*, *S. cuspidata*, &c.; or simply crenate, as in *S. erythropus* (Fig. 16), *S. serpens*, *S. involvens*, &c.; to a distinct fan-shaped body, also with crenate, lobed, or papillate margin, as in *S. grandis* (Fig. 14), *S. haematodes*, *S. caulescens*, *S. Karsteniana*, *S. viticulosa*, *S. plumosa*, &c. In *S. Martensii* (Fig. 13), perhaps the most perfect fan-shape is attained, the free distal margin being curved and about twice the breadth of the ligular base. Considerable

[1] In the naming of the species I have, as in previous papers, followed Baker's Fern Allies.

variation occurs in the precise form of the ligule, even in the same plant; but, on the whole, within certain limits, the outline is maintained fairly constantly for the species. In *S. Vogelii* (Fig. 15), *S. Griffithii*, *S. uncinata*, &c., the ligule is tongue-like and slightly crenate, without papillae.

The ligule is sunk in the tissue of the leaf-base by a more or less massive glossopodium, bearing an intimate relation to the cells of the leaf-base. In *S. haematodes*, for example, the glossopodium is scarcely at all sunk, whilst in *S. helvetica* (Fig. 17), *S. laevigata*, var. *Lyallii* (Fig. 21), and others, the ligule has a deep and well-marked glossopodium fitting into a distinct cup in the leaf-base. In all the species which I have examined, the glossopodium is enclosed by a distinct sheath of cells which are obviously continuous with the epidermal cells—on the one side, of the leaf, on the other, of the stem—and which are either cubical or elongated in the plane of the long axis of the leaf. These cells, when the ligule reaches a certain age, become strongly cuticularized and thick-walled. The base of the ligule itself, or glossopodium, is composed of specially large and clearly-marked cells with little contents. Where there are two rows of these, they are not unlike right triangular prisms with the long faces towards the sheath. The glossopodium as a whole may be looked upon as a blunt wedge, thick in the middle and thinning away rather abruptly to either margin. The wedge at its thickest part may be two, three, four or even more cells thick. Thus in *S. Martensii*, *S. grandis*, *S. Vogelii*, *S. Griffithii*, &c., there appear generally two such basal cells in any given section taken in the median longitudinal plane of the leaf; in *S. Douglasii*, *S. Karsteniana*, *S. viticulosa*, *S. helvetica*, &c., three such cells appear, the median cell being cubical or brick-shaped; whilst in *S. Braunii* there are four or even more such cells (Fig. 12). The primary number does not seem to me to be in all cases maintained, secondary divisions occurring as the ligule grows older. For instance, in *S. spinosa* the ligule has three initial cell-rows, which may, however, by division become four or even more (Figs. 1, 2, 4, 6).

Following upon the glossopodium comes a thicker swollen region whose cells are large, polygonal, and filled with dense granular protoplasm, with well-marked nuclei (Fig. 8). The long axis of the glossopodium is not in the same plane with that of the long axis of the body of the ligule, but cuts it at a more or less pronounced oblique angle, the glossopodium pointing away from the stem into the base of the leaf, while the free portion of the ligule lies almost parallel with the leaf-blade. The distal half of the ligule consists of smaller and less granular cells, and thins off to the free margin, which is unilamellar in the adult condition, the cells there being more or less filled with a granular slime. I purpose referring to these cells later on.

Between the glossopodial sheath and the leaf-trace-bundle there lie one to several layers of large cells, which radiate from the glossopodium outwards towards the leaf and inwards towards the stem. In most cases these cells retain their cellular character, or at most become slightly thickened and pitted; but in many they become markedly thickened, and in the mature condition are transformed into short, often branched tracheides. In *S. apus*, *S. stenaphylla*, *S. Martensii*, and others, only a few such secondary tracheides with scalariform markings are formed, so that the leaf-trace appears only slightly enlarged just beneath the glossopodium; but in *S. helvetica* these tracheidal elements are so numerous that the whole glossopodium is enclosed in a distinct cup-like vascular enlargement. An intermediate condition may be seen in such a form as *S. Wildenowii*, whilst *S. laevigata*, var. *Lyallii*, shows the greatest development of tracheides of any of the species I have examined. In that species (Fig. 21) a very distinct vascular cup is formed, which shows up very clearly if the ligule be carefully pulled out of its socket after boiling the portion of the shoot selected in dilute potash. This curious expansion of the leaf-trace, if it may be so termed, has already been noted by McNab (*l. c.*), and he doubtless based his theory of the absorptive function of the ligule on this fact. I think, however, that the phenomenon in question

is capable of another interpretation, which perhaps more satisfactorily meets the case.

The sheathing-cells of the glossopodium vary much in shape. Most frequently, as in *S. grandis*, *S. Martensii*, *S. caulescens*, *S. viticulosa*, &c., they are in the form of elongated staves or bars, curving round to meet at what may be termed the base of the foveola; whilst in other cases, e. g. *S. spinosa*, they are short cubes.

The ligule of *S. oregana* (Fig. 19) and *S. rupestris* deserves a word of explanation. In these species the free margin of the ligule scarcely appears above the edge of the very deep pit in the leaf in which it is seated. The vascular bundle of the leaf does not present any enlargement, but the radiating cells intervening between it and the sheath of the glossopodium are strongly sclerotic. The sheath consists of two rows of stave-like cells, and the glossopodium of two rows of right triangular prisms. The leaf-tissue, however, grows up and round the free portion of the ligule, so that a deep pit is formed, the cells of which have no connexion with the ligule. These appearances are illustrated in Fig. 19.

B. The Development of the Ligule.

In tracing the development of the ligule, I confined myself to the study of the shoots of two species, viz. *S. spinosa* and *S. Martensii*; partly because I had the advantage of comparing my own preparations with the large series kindly lent me by Professor Bower, and partly because, from an examination of the adult state of the ligule in numerous species, I felt that the differences in mode of development were unlikely to be very fundamental.

S. spinosa. The ligule, as in all the species of this genus, is multicellular in origin. If careful longitudinal sections be made of the growing-point, the ligule may be distinguished first about the level of the third or fourth leaf as a fairly distinct swelling immediately at the base of the leaf. The swelling consists of a short ridge about six or eight cells in length in the plane of the leaf-face, and three broad, that is to say,

in the plane at right angles to the ridge. Fig. 1 shows the earliest distinctly recognizable stage in the development, the left-hand side of the figure representing the surface of the stem, whilst the leaf-face is partly shown on the right. The ridge is shown here as three cells broad, and these cells may be distinguished very early, not only by their greater size, bulging out into the angle between the leaf and the stem, but also by the fact that they take on a deeper stain than do the surrounding cells, and have larger nuclei. At the margins the ridge is only one cell broad. These cells speedily undergo transverse division, so that a basal sheath of cubical cells (Figs. 2, 3, and 4) is formed continuous with the epidermis of the leaf and stem, the free segments growing outwards to form the ligule itself. The sheath-cells may undergo further divisions, as shown in Figs. 3 and 6, and the cells of which it is composed at a later date become thick-walled and cuticularized. The next layer of cells from the merismatic prominence gives rise to the glossopodium; these cells enlarge greatly, and do not stain so deeply as the sheathing-cells or those of the body of the ligule, whilst the general cells of the body of the ligule remain as a rule of much smaller size.

The ligule has completed its development long before the leaf to which it belongs; indeed, it has reached its adult size and shape at a distance of from 1 to 1½ mm. from the apex of the shoot. Occasionally the clearer basal cell-layer or glossopodium becomes four cells broad (Fig. 6), but three is by far the commoner number. The body of the ligule itself may, however, be six or even more cells thick. Fig. 5 shows a transverse section of the ligule in a comparatively young condition, while in Fig. 6 a longitudinal section of an adult ligule shows it to be composed of five cell-rows.

S. Martensii. Pfeffer (*l.c.*) describes the ligule of *S. Martensii* as arising from one row of from four to six cells. I have made careful examinations of longitudinal sections of very many growing apices, and find no evidence in support of his statement or figure. The ligule appears to me to arise invariably from two rows of cells (Fig. 7), which can easily be

distinguished both by their greater size and more granular and denser protoplasm, as well as by their conspicuous bulging into the angle between the stem and leaf. In this view I am supported by Bower's figures illustrating the development of the sporangium (*l.c.* Pl. 47, Fig. 79). As in *S. spinosa*, the two primary cell-rows undergo transverse segmentation, so as to isolate a set of sheathing-cells surrounding the foot of the ligule. The outer cell-segments then separate another set of large comparatively empty cells, which grow in size as the ligule develops. Rapid segmentation of the apical region then takes place so as to form a thick swollen portion, which later becomes two-layered, and finally ends in a unilamellar apical plate. In the ligules of the vegetative leaves the sheathing and the glossopodial cells remain in two rows, but in the ligules associated with sporangia both sheathing and glossopodial cells may divide so as to form three rows, at all events in the thicker median region of the ligule.

All the observations I have made on the development of the ligules of other species point to a similar embryonic history, and I feel convinced that the number of cell-rows in the adult glossopodium will give a fairly reliable indication of the number of primary merismatic cell-rows which take part in the formation of the ligule, though doubtless secondary divisions may occur in these, as I have already pointed out in *S. spinosa*.

C. The Function of the Ligule.

The homologies and functions of the ligules in the genus *Selaginella* have for long been matters of controversy. It has been suggested, for instance, that the ligule may be of the nature of an indusium, a view against which many and obvious objections may be raised. McNab (*l.c.*) considered it as an organ of absorption, basing his conclusion on the close association of the ligule with the vascular bundle of the leaf. A homology between the ligules of *Selaginella* and of *Isoetes* has been made much of by many writers, and has, as is well known, been employed as a basis of classification; but, as

S. Martensii, Spr.

Fig. 7. Longitudinal section through the growing-point of *S. Martensii*. ×350. The initial cells of the ligule are shaded.

Fig. 8. Longitudinal section through an almost mature ligule. The narrow sheathing cells, three in number in section, are followed by three glossopodial cell-rows; the body of the ligule is composed of an irregularly arranged mass of densely granular cells, followed by the membranous terminal lamella. ×350.

S. Braunii, Bak.

Fig. 9. Longitudinal median section of a very young ligule of *S. Braunii*. Four rows of initial cells are represented. ×350.

Fig. 10. Longitudinal section of a slightly older ligule of the same. ×350.

Fig. 11. Transverse section of the base of a young ligule, taken just above the glossopodium. ×350.

Fig. 12. Longitudinal section of an old ligule. The sheathing and glossopodial cells are arranged in four rows. ×350.

Fig. 13. Ligule of *S. Martensii*, Spr., isolated from its sheathing-cells. ×60.

Fig. 14. Isolated ligule of *S. grandis*, Moore. ×60.

Fig. 15. Isolated ligule of *S. Vogelii*, Spr. ×60.

Fig. 16. Isolated ligule of *S. erythropus*, Spr. ×60.

Fig. 17. Longitudinal median section through an old ligule of *S. helvetica*, Lk., showing the thick-walled sheathing cells and the enlargement of the vascular bundle of the leaf to form a tracheidal cup round the glossopodium. ×350.

Fig. 18. Transverse section through the glossopodial region of an old ligule of *S. flabellata*, Spr. ×350.

Fig. 19. Longitudinal median section through the ligule and leaf-base of *S. oregana*, Eat. ×150. There are two rows of sheathing and of glossopodial cells, the entire ligule being depressed and almost hidden in an involution of the leaf-base.

Fig. 20. Longitudinal median section of the base of a mature ligule of *S. grandis*, Moore. ×350.

Fig. 21. Longitudinal median section of the ligule of *S. laevigata*, Bak. var. *Lyallii*, Spr., showing the enlarged tracheidal cup surrounding the base of the ligule. ×150.

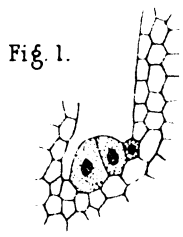
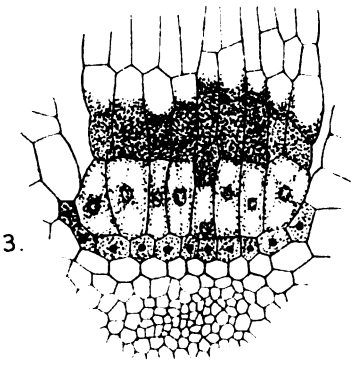


Fig. 1.



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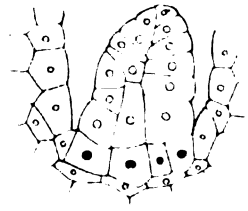

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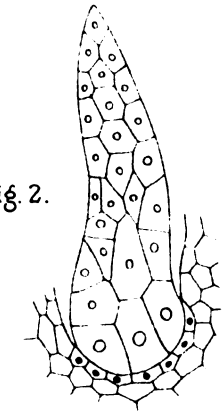


Fig. 2.

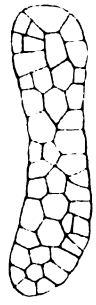
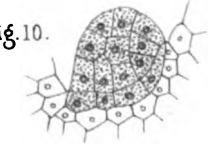


Fig. 5.



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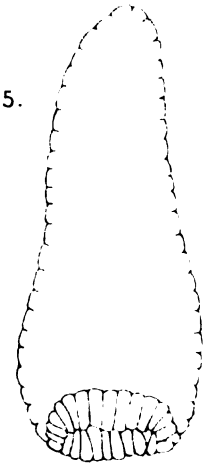

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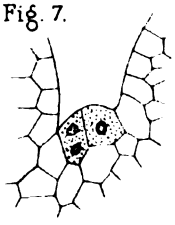

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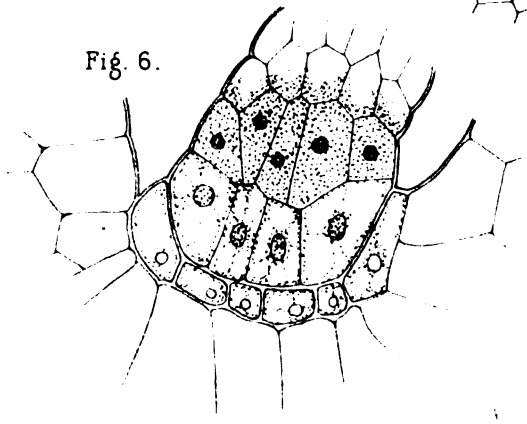

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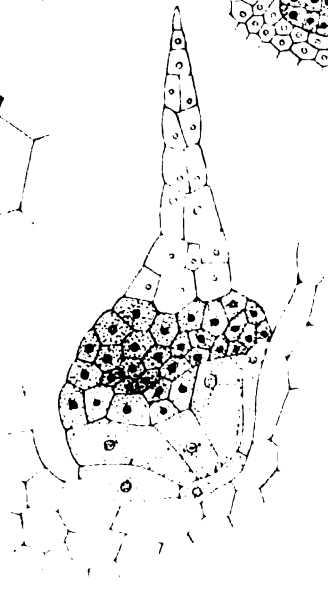

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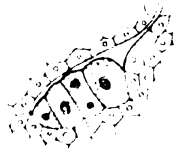

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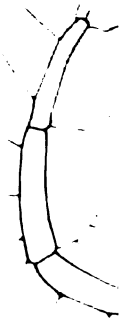

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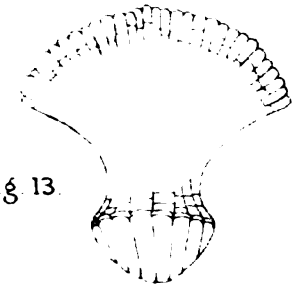
g. 13.



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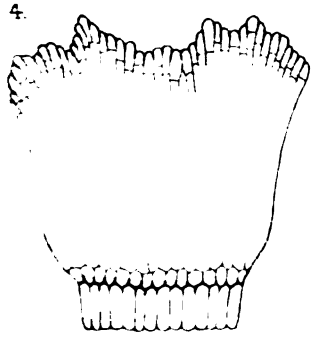

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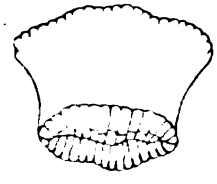

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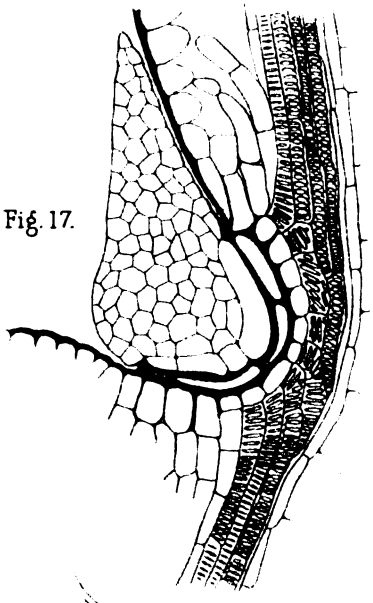

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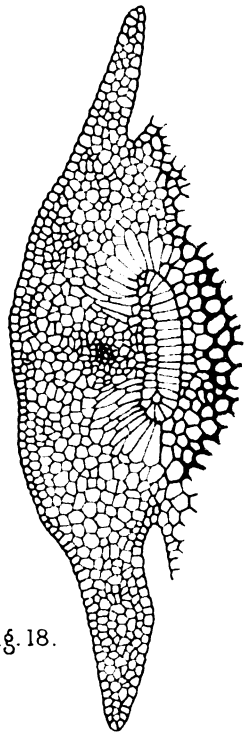

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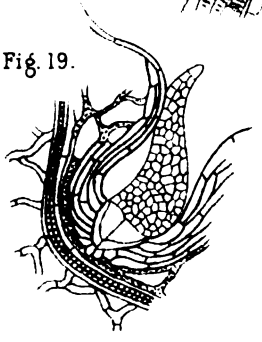

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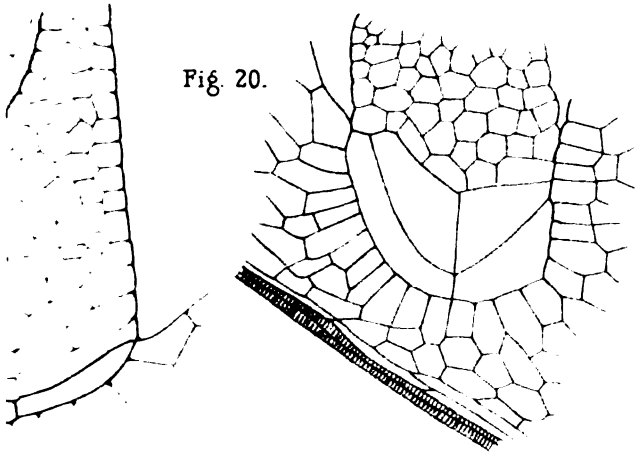

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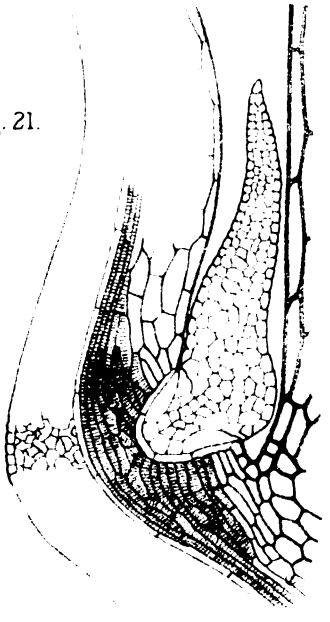

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NOTES.

REPRODUCTION AND FERTILIZATION IN CYSTOPUS CANDIDUS.—In a paper read before Section D at the Edinburgh meeting of the British Association in 1892, on the structure of this Fungus, I gave a short account of the cytology and development of the asexual and sexual reproductive organs. This paper was incomplete and in part somewhat incorrect; but since that time I have obtained fresh material and am now able to give a much fuller account, which will be published shortly in the Annals, and of which the following is a summary. The literature on this subject is not very extensive. Among those who have paid special attention to it are Fisch, Zalewski, Dangeard, and Chmielewsky. The results obtained by these observers are fully discussed in the complete paper.

The Fungus is found as a parasite on the leaves, stem, and ovaries of various cruciferous plants, especially the common Shepherd's Purse (*Capsella Bursa-Pastoris*), on which it forms white shining patches, often of considerable size. The mycelium consists of non-septate hyphae, which ramify in all directions between the cells of the host-plant, produce small spherical hustoria, which penetrate the cells, and under certain conditions cause hypertrophy of the organs attacked. The mycelium is at first found only in the superficial layers of the plant, but at a later stage it penetrates into the deeper layers, and even into the pith. In the earlier stages of development this mycelium produces club-shaped branches just beneath the epidermis, from which the asexual organs (spores or sporangia) are delimited; and at a later stage sexual organs (antheridia and oogonia) are produced on the ramifying mycelium, sometimes intercalary, sometimes terminal. The protoplasm of the hyphae is vacuolate, and consists of a loose network in which are to be found numerous small nuclei. In certain parts of

same time new lateral buds might be shooting out. These considerations led Schimper to conclude that calcium plays no fundamental part in the conduction of carbohydrates.

This conclusion still left unexplained the abnormal accumulation of starch in plants cultivated in solutions devoid of calcium. It appeared to me that the explanation might be very simple, namely, that though potassic oxalate did not prevent the transport of sugar, it was capable of arresting the change of starch into sugar. It naturally suggested itself that acid potassic oxalate should either prevent diastase from being formed or should stop its action. I therefore made some experiments on the influence of acid potassic oxalate on diastatic action.

In these experiments a strong solution of 'extract of malt' was used in place of pure diastase, to act upon a clear portion of a ·5 per cent. solution of arrowroot-starch. Bacteria were kept at bay by means of traces of thymol. In each case 10 cc. of starch-solution were mixed with 10 cc. of certain strengths of acid potassic oxalate solution; then 10 cc. of the solution of 'extract of malt' were added. The following table shows the result of experiments conducted at a temperature of about 30° C, and commenced at 11 a.m.

Solutions.	Resulting percentage of acid potassic oxalate in the whole mixture.	Colour when treated with iodine at		
		12 a.m.	1.30 p.m.	4.30 p.m.
A. B. C. D.	·6, ·5, ·3, ·26	Blue	Blue	Blue
E.	·1⅓	Dirty brown	Lighter brown	Yellow
F. G. H. K.	·04, ·02, ·01⅓, 0	Yellow	Yellow	Yellow

This preliminary set of experiments roughly showed that the action of diastase is retarded by even dilute solutions of acid potassic oxalate. I subsequently found that similar rough observations had been made by Detmer, whose investigations are quoted in Schleichert's Das diastatische Ferment der Pflanzen. Quantitative observations, in which pure diastase was employed, have been conducted by Mr. J. H. Manley, of the Magdalen College Chemical Laboratory, Oxford; these, though not yet complete, confirm the rough observations.

It became essential to see next if this soluble oxalate had the same action on starch in the living leaf. For experiments I selected sub-

merged aquatic plants, in order that salt-solution should penetrate the living cells rapidly. After some rough preliminary experiments on *Vallisneria*, I selected *Elodea canadensis* and *Callitriche* sp.

Experiments with Elodea.

The leaves were placed in 50 cc. of solutions *A* to *L*, varying therefore from 0 per cent. up to ·24 per cent. acid oxalate of potassium. When first put in the leaves contained starch. The leaves of *Callitriche* were only casually examined to see that there was in general the same distribution of starch. The following table represents under the general heading II, the amount of starch found in leaves kept darkened for 24, 72, and 96 hours, in solutions *A* to *L*.

	II.			III.
Strength of solution of acid potassic oxalate.	Amount of starch in leaf after darkening for 24 hours.	72 hours.	96 hours.	Amount of starch in leaves after several days' illumination. Examined sometimes between 10 a.m. and 1 p.m.
A 0	0	0	0	Starch present.
B ·000065	0	0	0	,,
C ·00025	0	0	0	,,
D ·001	0	0	0	More starch than in *C*, *B*.
E ·0025	0	0	0	Much starch: more than *B*, *C*, *D*.
F ·005	Small amount	0	0	,, ,,
G ·01	,,	Small amount	Minute amount	If anything had less starch than before illumination.
H ·04	?	Considerable amount	Considerable amount	Considerable amount of starch.
I ·08	Considerable amount	,,	,,	,, ,,
K ·16	,,	,,	,,	,, ,,
L ·24	,,	,,	,,	,, ,,

These observations show that acid potassic oxalate retards the process of the change of starch into sugar in the living leaf.

It remained to be seen, if, under conditions of alternating light and darkness, an abnormal accumulation of starch could be induced, such as occurs when plants are cultivated in solutions devoid of calcium. To ascertain this I continued this second series of experiments, but placed the leaves, still lying in their respective solutions, in front of a window in the laboratory. They were allowed to remain thus for

two or three days[1]. The last column (96 hours) under the heading II of the foregoing table, therefore represents the amount of starch contained by these leaves before illumination; and the details given under the heading III describe how much starch there was after two to three days and nights. In *A*, *B*, *C* there was some starch, showing that starch was being formed but was constantly being reconverted into sugar. In *D* there was more starch, and in *E* and *F* much more starch: thus showing that starch was being manufactured but that its reconversion into sugar was slower than in *A*, *B*, *C*. In *G* to *L* there was no appreciable change in the amount of starch caused by illumination: thus demonstrating that not only was the change of starch into sugar retarded but that the manufacture of starch was almost or quite arrested.

Restoring all the leaves into tap-water showed that those which had been in *A*–*G* were still alive and could make starch, whereas those in *H*–*L* had been killed.

The difficulty at once presents itself as to how the change from starch to sugar is accomplished in leaves which normally contain a considerable amount of acid potassic oxalate in solution. I therefore made several series of experiments with leaves of *Oxalis floribunda*, in which there is a large amount of soluble oxalate. A description of one of these series will suffice to show the results obtained.

Experiments with Oxalis floribunda.

Leaves were picked which contained a considerable amount of starch. The leaflets were pulled from the petiole and then either cut transversely or longitudinally into two halves, or they were only wounded by three cuts extending from the margin of the distal or proximal part towards the centre of the leaflets. These leaflets were then dropped into solutions *A*–*L* identical with those used in the *Elodea*-experiments, and into an additional solution (*M*) of ·5 per cent. acid potassic oxalate. The cuts were made in order to allow the solution to penetrate the leaves. The leaflets in the respective solutions were then kept in darkness for ninety-six hours, at the conclusion of which they were examined. Leaflets in *A*–*E*, cut in various ways, all showed starch only in the actually injured cells at the cut surface. Leaflet in *F* had starch only in a narrow band of cells bounding and running

[1] I unfortunately omitted to record the number of days.

parallel to the cut. Leaflets in *G–M*, cut in various ways, all possessed starch in a band of cells bounding and running parallel to the cut. In *G–M* the band was broader than in *F*. Elsewhere there was no starch excepting a small amount coating the vascular bundles for a short distance as they radiated from the cuts.

These results prove that in *Oxalis* a dilute solution of acid potassic oxalate in the assimilating cells will prevent the starch from being changed into sugar. Yet during the normal life of this *Oxalis* there is a large amount of the oxalate, still no such accumulation of starch ensues. For the explanation of the mystery we must look to the distribution of the acid oxalate in the normal leaves of *Oxalis*. Giessler (4) showed that the soluble oxalate is stored in the epidermis of the leaf, not in the assimilating tissue. The oxalate is stored in the epidermis because its presence in the mesophyll would derange the metabolic processes; and not because the plant needs to protect itself against snails, &c., as Giessler suggests. The protective significance of the superficial distribution of the oxalate is at most secondary.

To sum up:—

(1) Acid potassic oxalate retards the action of diastase on starch.

(2) In the living plant the first, and, at the commencement, the only visible effect of acid potassic oxalate on the assimilating organs is the accumulation of starch, owing to an arrest of the change of the starch into sugar.

(3) The second effect, as the soluble oxalate accumulates, is a retardation of the manufacture of starch, and hence probably of the assimilation of carbon.

(4) The last effect, with increased accumulation of the oxalate, is the death of the protoplasm.

These researches therefore confirm the discovery made by Schimper, that the evil effects of a lack of calcium are to be attributed to the accumulation of acid potassic oxalate in plants which normally contain calcic oxalate. They may be regarded as complementary in showing that, in the absence of calcium, there is a stoppage of the conduction of those carbohydrates only which have entered into the condition of starch. Schimper (5) showed that part of the carbon assimilated by a plant never enters into the starch-condition, and this has been confirmed by Brown and Morris (6). It is therefore clear why growth is not at once arrested in shoots or seedlings deprived of calcium.

LITERATURE.

(1). J. BOEHM: Ueber der vegetabilischen Nährwerth der Kalksalze. Sitzber. d. Wiener Ak. d. W. Bd. 71, 1875.

(2). A. F. W. SCHIMPER: Ueber Kalkoxalatbildung in den Laubblättern. Bot. Zeit. 1888 (Sep. Abd.).

(3). A. F. W. SCHIMPER: Zur Frage der Assimilation der Mineralsalze durch die grüne Pflanze. Flora, 1890 (Sep. Abd.).

(4). R. GIESSLER: Die Localization der Oxalsäure in der Pflanze. Bot. Zeitung, 1894, No. 2, p. 25.

(5). A. F. W. SCHIMPER: Ueber Bildung und Wanderung der Kohlhydrate in den Laubblättern. Bot. Zeit. 1885 (Sep. Abd.).

(6). HORACE T. BROWN & G. H. MORRIS: A Contribution to the Chemistry and Physiology of Foliage-leaves. Abstract in Annals of Botany, VII, 1893, p. 271.

PERCY GROOM.

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NOTE ON SACCORHIZA BULBOSA, J. G. AG., AND ALARIA ESCULENTA, GREV.—In his paper on the structure and development of the bulb in *Laminaria bulbosa*, Lam. in Vol. III of the Annals of Botany (pp. 41–64), Barber gives an interesting *résumé* of the literature dealing with this plant. From the references there given, it would not appear that the duration of the plant had, at that time, been satisfactorily settled. From observations made last year while collecting Algae on the coast of Anglesey, I have satisfied myself that the species is an annual, and in this respect contrasts sharply with the other British Laminariaceae.

During the spring tides of March, I had an opportunity of examining *Saccorhiza* growing, and at that time, I found it represented by specimens in two widely different conditions. First, there were large bulbs often more than a foot in diameter, from which the whole of the blade, and almost the whole of the stalk had disappeared through decay. The bulbs themselves were as yet sound, and required the exertion of considerable force to remove them. Among these blackish masses, bristling with 'hapteres,' were numerous young plants of a lighter colour, and exhibiting the various stages in the development of the bulb described by Barber; but in no case had the bulbous portion attained a diameter of more than four inches, or the whole plant a length of more than two feet. Upon visiting the same site during the spring tides of October, I could find no trace of exfoliated bulbs or of young imperfectly developed plants. All the specimens were now in the condition of the mature plant, with which we are made

familiar in Harvey's figure (Phycologia Britannica, pl. 241). It would thus seem that the young plants of March were the progeny of the plants of the previous autumn, maturing in the next autumn, to remain however, as exfoliated bulbs as late as March of this year. The plant is therefore an annual in the sense that the plants of one year produce the crop of the next; in the same sense, that is to say, as winter-sown wheat is an annual.

I have since ascertained that Setchell, in a paper on the Life-History of *Saccorhiza dermatodea* (De la Pyl.) J. G. Ag. in the Proceedings of the American Academy, Vol. VI, expresses himself as convinced of the annual character of that species, and states that De la Pylaie and Areschoug had come to the same conclusion. It would thus seem that two of the species removed from the genus *Laminaria* into De la Pylaie's genus *Saccorhiza* turn out to be annuals.

The absence in *Saccorhiza bulbosa* of the intercalary growth, which in British Laminarias forms a new lamina each year, the non-occurrence of a zone of secondary thickening in the stipes, as well as a softer consistency of the whole substance of the plant, are all to be correlated with the fact of its lasting for a single season. The persistence of the bulb for some time after the disappearance of the stalk and blade, adds a new interest to Gardiner's[1] discovery of sporanges upon the 'roots.'

In the same paper by Barber as is referred to above, when speaking of the annual shedding of the lamina in the Laminarias, he remarks that 'it is stated by Reinke to occur also in *Alaria esculenta*.' It may fairly be concluded, I think, from the bright olive colour and entire condition of the proximal portion of the blade, when contrasted with the darker colour and frayed condition of the distal portion, that an intercalary growth takes place in *Alaria* in the same region as in the Laminarias. This appearance however persists throughout the year, and I have not seen any evidence of a similar insertion of an entirely new lamina in the spring as occurs in *L. digitata* and *saccharina*. The intercalary growth in *Alaria* seems to be continuous; in *Laminaria*, periodic. It is interesting to notice that young specimens of *Alaria* not more than two or three inches long exhibit the same fresh appearance of the near end, and frayed appearance of the free end as occur in specimens several feet long.

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[1] Proc. Camb. Phil. Soc., V, 1885.

THORNS WITH CORKY BASES—A CORRECTION.—I am desirous to publish the following correction to my list of plants having thorns with corky bases, contained in a note by Dr. Trimen of Ceylon, which, although dated January 16, 1893, has only just reached me:—

'At p. 165 of the sixth volume of the Annals of Botany, among the plants recorded by Mr. Barber as having corky thorns, is *Ailanthus malabarica*, which is entered on the authority of a photograph taken at Peradenyia by Mr. Potter. The name is erroneous, the tree being *Zanthoxylum Rhetsa*. But I believe that at the time the photograph was taken, the plant had by some accident a wrong label attached to it.'

Zanthoxylum Rhetsa is already in my list; and, as *Ailanthus malabarica* is the only example quoted of Simarubeae, that order will have to be ruled out. Many additions might doubtless be made by travellers in the tropics with the means of identifying forest-trees. The only case I could be certain about in the Leeward Islands is the common sand-box tree, *Hura crepitans*, which has thorns with stony bases exactly like those on the specimen of *Erythrina lithosperma* in the Botanical Museum at Cambridge.

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THE CYTOLOGY OF SAPROLEGNIA.—The almost simultaneous appearance of Mr. Trow's interesting and elaborate paper on the 'Karyology of Saprolegnia' in the Annals of Botany for December, 1895, and of mine 'On the Cytology of the Vegetative and Reproductive Organs of the Saprolegnieae' in the Transactions of the Royal Irish Academy (read February 19, 1893, revised for press June, 1895), renders it desirable that I should make some remarks on the points at issue between us.

My first and gravest criticism on matters of fact is that Mr. Trow has overlooked the existence of four chromosomes in the vegetative nuclei, to which he ascribes only a single chromosome; he admits that his 'observations on this point might well have been more extensive' (p. 624)[1]. Considering the importance he attaches to the

[1] The context in my first publication on this subject (Comptes Rendus, CVIII, 1889) would seem to render it plain that my observations on nuclear division referred to the vegetative hyphae. But Mr. Trow writes 'as he expressly states that divisions do not occur in the oogonia, I conclude that he observed these phenomena in the antheridia' (p. 629)—a curious non-sequitur.

number of these nuclear organs, we must regret this; all the more since his deductions have seriously influenced his interpretation of the processes in the oangium.

His *observations* on this second point have evidently been numerous and careful; and indeed they afford a welcome confirmation to my own. But in his *interpretation* of the different appearances of nuclei in the same oangium, and in his assumption that the majority are practically digested by the cytoplasm, I cannot follow him. I must still hold that the divergence of character is due to the various number of nuclei that have fused, and to the varying stages of the fusion in the different nuclei as their number is reduced by this fusion.

We next come to the question of fertilization by the antheridial tubes: of this alleged phenomenon I found no positive evidence whatever, but every indication to the contrary, as is fully stated in my complete paper. I have indeed found and figured (T. xxix, fig. 25) a young oospore of *Saprolegnia* with two nuclei which I ascribe to retarded fusion, as is so frequently the case in *Achlya*; but in this case there was no antheridium present whatever, while many hundreds of oangia with attached antheridia and contained antheridial tubes did not supply a second case of a binucleate oospore in this genus.

The general conclusions of the paper are largely based on the assumption that Weismannism is scientifically demonstrated; but as I am not prepared to admit this as a common basis of argument, I shall abstain from discussing these conclusions. I must, however, point out that it is an anachronism in 1896 to make the following statement: 'In the theory of heredity so brilliantly propounded by Weismann, the admixture of the substance which is the bearer [sic] of the hereditary tendencies during the sexual process is looked upon as the chief cause of variation in the higher organisms' (p. 643). This was indeed the view of Weismann in 1891, till it was demonstrated in a theorem, rejected at first by his disciples but admitted as 'a logically correct deduction' by himself, that the theory of 'Amphimixis' as the chief cause of variation was in contradiction either with itself or with the facts of nature. Weismann having after this, to use his own words, 'gained a deeper insight into the problems concerned,' published in the 'Germ-Plasm' (London, 1893) the following statements duly italicized:—'*Amphimixis . . . . is not the primary cause of hereditary variation*' (p. 414): '*the cause of hereditary variation must lie deeper than this, it must be due to the direct effect of*

external influences on the biophors and determinants' (p. 415). *'These very minute fluctuations [i.e. continual changes of composition in the elements of the germ-plasm] which are imperceptible to us, are the primary cause of the greater deviations in the determinants which we finally observe in the form of individual variation'* (p. 417).

Finally, I must express my regret that so careful a technique and such powers of observation as the author's should have been overweighted with *a priori* and obsolete views. It is not good to follow too literally the adage 'Eher eine *schlechte* Hypothese als gar keine.'

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Feb. 14, 1896.

STUDIES IN THE MORPHOLOGY OF SPORE-PRODUCING MEMBERS. PART II: OPHIOGLOSSACEAE[1].— In a paper published in the Philosophical Transactions (Series B, 1894), the comparative study of the spore-bearing members of the Lycopodineae, including the Psilotaceae, has led to the conclusion that there is reasonable probability that septation of sporangia originally simple, to form synangia, has taken place; that a septate body (synangium) may be homologous with a non-septate body (simple sporangium); and that there is no essential difference between tissue which will form septa or trabeculae, and that which will form spores, since the tissues can mutually undergo conversion one into the other.

But the considerations there brought forward do not amount to an actual demonstration that septation has occurred. For the purpose of our discussion, it is important to ascertain whether such demonstration can be given in the case of parts which are undoubtedly homologous; it is afforded by the study of septate anthers, which occur in several distinct families of Angiosperms, e.g. Mimoseae, Onagraceae, Loranthaceae, Myrsineae, Rhizophoreae, Orchidaceae, Rafflesiaceae. Taking the case of the Onagraceae, the common type of the anther is the ordinary quadrilocular type, but in certain genera transverse septa are formed in each of the four pollen-sacs by conver-

[1] Read before the Royal Society, Dec. 5, 1895: reprinted by permission from Proc. Roy. Soc., No. 354, Vol. lix.

sion of certain cells of the potential archesporium into sterile tissue; the unavoidable conclusion already drawn by other writers for this and other cases, is that these septate anthers are derived from those of the ordinary quadrilocular type, and the process of septation thus illustrated is essentially similar to that described for the Lycopodineae in my previous paper. *We thus see that septation of sporangia has actually occurred, and that it is a rather wide-spread phenomenon in Angiosperms.* It will therefore be merely a question of probability whether, and how far, it has also occurred in lower forms, and whether it is by septation that those synangia were produced, which are so marked a feature in certain Pteridophyta.

The argument from developmental evidence is comparatively simple where, as in the Angiosperms, the meristems are distinctly stratified, and the archesporium is a definite layer, ultimately hypodermal in origin; but in applying a similar argument to the Pteridophyta, in which the meristems are not clearly stratified, it is less easy to arrive at a conclusion. The principle is to be laid down that *the study of the sporangia or synangia of a plant is to be carried out in the light of a knowledge of the segmentation of its apical meristems.* The sporangia are parts of the plant-body, and their segmentations do not differ essentially from those of the meristems of the other parts of the plants on which they occur. Where the meristems are stratified, as in Angiosperms, a clearly stratified structure of the sporangia is commonly found; where, as in the Pteridophyta, the meristems are not stratified, it would be plainly unreasonable to expect a stratified structure of the sporangia, and such structure is not found. Accordingly, in using developmental evidence in solving the question whether synangia in Pteridophytes resulted from septation, the existence of a continuous hypodermal archesporium cannot reasonably be demanded as evidence of septation, though of course it may occur, as indeed it does in *Isoetes*; it is, however, to be remembered that in this plant the meristems are more clearly stratified than in most Pteridophyta.

The considerations thus briefly epitomized are a necessary prelude to the comparative study of the Ophioglossaceae. In my preliminary statement (Roy. Soc. Proc., Vol. l, p. 265) I have described, chiefly from examination of *Ophioglossum pendulum*, a continuous hypodermal band as the potential archesporium, which subsequently was differentiated into sporogenous groups and septa; such a band appears

with some degree of regularity in this species, but it is not constant, and is not found with any clearly defined outer limit in *O. vulgatum* or *reticulatum*; thus far I admit the validity of Rostowzew's criticism of my preliminary statement (Beitr. z. Kenntniss der Ophioglosseen. 1. *Oph. vulgatum*, L., Moscow, 1892). On looking more carefully into this question, however, I have found that a band of superficial cells, differing in origin and segmentation from the surrounding cells, may be recognized as the *sporangiogenic band*; this gives rise to the sterile septa, the sporogenous groups, and the external wall of the sporangia; the band usually consists of two longitudinal rows of cells, possibly referable in origin to a single row, but there is some variety of detail. The observations have been made on three species, viz. *O. vulgatum*, L., *O. reticulatum*, L., and *O. pendulum*, L.

The band, at first undifferentiated, subsequently forms (i) archesporia at intervals, (ii) sterile septa which intervene between them, and (iii) the outer sporangial walls. The cell-groups which give rise to septa and to archesporia are sister cell-groups, having a common origin, and no difference can be seen between them in early stages; the distinction only becomes apparent as the archesporia attain their characteristic denser contents, and the difference is thus functional, not genetic. The archesporium of the single sporangium has not been found to be referable in origin to a single cell, and it is not defined by the first periclinal wall of the cells of the sporangiogenic band. These facts are all compatible with a theory of the origin of the spike of *Ophioglossum* by septation from a simple sporangium of the Lycopodinous type, and the sporangiogenic band may be compared with the band of cells, sometimes a single row, or two or three, which, after periclinal division, give rise to the archesporium of *Lycopodium*.

The development of the sporangia of *Botrychium* and *Helminthostachys* has also been traced, but these facts do not bear so directly upon the question of the nature and origin of the Ophioglossaceous spike as those derived from the study of *Ophioglossum*.

Abnormalities have played a large part in former discussions upon the morphology of the spike in the Ophioglossaceae. While recognizing the obvious correlation which exists between vegetative development and spore-production, it has been concluded that the abnormalities in this family do not form a sufficient basis for argument, certainly not when the conclusions drawn from them are in opposition to the

results of comparison of normal specimens. Such comparison led Mettenius, Strasburger, Celakovsky, and others to recognize a relationship of the Ophioglossaceae to the Lycopods. This comparison has been developed at considerable length, on grounds not only of the similarity of the development and position of their spore-bearing members, but also by comparison of the synangia of the Psilotaceae; the Gametophyte, also, and sexual organs and embryology, as far as known, have been taken into account, and a detailed comparison made of certain features in the anatomy of the Lycopods and Ophioglossaceae. From these various sources a general support of the relationship has been traced, the nearest point of comparison appearing to be between *O. Bergianum* and *Phylloglossum Drummondii*; it is contended that this is not a case of mere mimicry, but of real relationship, though such relationship probably dates from a remote and unknown ancestry.

Such a relationship would involve the idea of septation of the simpler type of Lycopodinous sporangium, to form the spike of *Ophioglossum*, but it has been shown that septation of a very similar nature has occurred in the anthers of certain Angiosperms, and that the developmental details of *Ophioglossum* are compatible with such a view. The conclusion of Celakovsky is, therefore, regarded as probably true, viz. 'that both the Lycopodiaceae and Ophioglossaceae sprang from a common stock, which had the simple sporophylls of the Lycopodiaceae. The Lycopods are probably, of living plants, the nearest prototypes of the Ophioglossaceae.' Thus, the view put forward is not new nor original, but, being now based on a wider area of fact, may take rank as a reasonably probable theory.

A comparison of the Ophioglossaceae among themselves shows that probably the genus *Ophioglossum* forms a series of increasing complexity, extending from such types as *O. Bergianum* or *lusitanicum* to such forms as *O. pendulum* and *palmatum*. Comparison of a large number of specimens of the latter species shows that the many-spiked condition is led up to by specimens with one, two, or three spikes, which are matched by abnormal specimens of *O. vulgatum*. The view is put forward that the many-spiked condition occasionally met with in other species has become the typical state in *O. palmatum*, and that it has been brought about by a chorisis or interpolation similar to that of the stamens of certain Angiosperms. It is further to be added that the insertion of the spikes is commonly on the

adaxial surface of the frond, rarely upon the margin; the facts accordingly do not support the hypothesis that the many spikes are of the nature of pinnae: thus, in *Ophioglossum* the progression appears to be towards multiplication of sporangia and formation of a plurality of spikes.

In *Botrychium* the progression appears to be from types such as *B. simplex*, in which there is close similarity to a simple *Ophioglossum*, by branching of the spike which is closely connected with enlargement and septation of the sporangia, to the condition seen in such species as *B. virginianum*, the branching of the spike running parallel with that of the subtending frond. The formation of sporangia abnormally on the latter, a condition commonly seen in *B. Lunaria*, but rare in most other species, is believed to be an example of reversion of a part typically vegetative to the sporogenous condition, and not indicative of a common character of the spike and the vegetative frond. Finally, *Helminthostachys* occupies an interesting intermediate position; the replacement of the sunken sporangia of *Ophioglossum* by projecting sporangiophores in *Helminthostachys* suggests, as already indicated in the preliminary statement (Roy. Soc. Proc., Vol. l, p. 265), an interesting analogy with the hypothetical origin of the strobilus of *Equisetum* from a body of the nature of a sporogonial head.

The chief object in view in these investigations has not been the mere tracing of homologies of parts among living forms; but, by developmental study and comparison, the following out of the probable methods of progression in the evolution of the more complex from the simpler types. It is believed that all the three methods of increase in number of separate sporangia, suggested in the former memoir (Phil. Trans., 1894, Vol. B, p. 473), have been employed, viz. (i) septation, (ii) branching or chorisis, (iii) a reversion of vegetative parts to the sporogenous condition. In addition to these, however, there has probably occurred also an eruption of appendicular organs from a previously smooth surface. This has already been suggested elsewhere (Annals of Botany, Vol. viii, p. 343); the sporangiophores of *Helminthostachys* may be taken as an interesting example of such eruption. It will be thus seen that the memoir, of which this is a very brief abstract, touches some of the most fundamental conceptions of the morphology of vascular plants, approaching them, not from the point of view derived from comparison of higher forms,

but from the study and comparison of organisms which are believed to be nearer to the border line between Bryophyta and Vascular plants, viz. the *Homosporous Pteridophyta*.

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PRELIMINARY STATEMENT ON THE SORUS OF DANAEA[1]. The sorus of *Danaea*, though its structure in the mature state has been repeatedly described, has not yet been studied as regards its development.

The oblong, cake-like sori lie parallel to one another on the lower surface of the leaf, their longer axes following the course of the vascular bundles. Each sorus consists of two rows of loculi, of approximately equal size, completely sunk in the rather massive tissue of the wall. Dehiscence is described as being by a pore at the apex of each loculus.

The sporogenous tissue of each loculus is usually referable to the segmentation of a single superficial cell, which gives rise to it, and to the portion of the sporangial wall above it. There is, however, great variety of bulk, number of cells, and mode of segmentation in adjoining sporangia; though the sporangia of the same sorus develop simultaneously, such extreme differences of number as between four and sixteen cells may be seen in the same section. Moreover, the whole sporogenous group is not always referable to a single parent cell. These facts stand in marked contrast to the uniformity of size and segmentation so characteristic of the Leptosporangiate Ferns.

The dehiscence is by slits, which appear in the sporangial wall above the loculus. By the drying and contraction of the adjoining cells the slit gapes widely, and appears as a pore; but the details are very like those of other Marattiaceae, excepting that there is no 'annulus' of indurated cells. This, which is absent in *Danaea*, where the loculi are deeply sunk, is present in those Marattiaceae in which the sporangia project as separate papillae. In the latter case the annulus is mechanically effective in widening the slit; in the former, the sporangia being closely united, such mechanical effect would be impossible.

Though the loculi of a sorus are frequently of nearly uniform size,

[1] Read before the Royal Society, Dec. 5, 1895: reprinted by permission from Proc. Roy. Soc., No. 354, Vol. lix.

examples may often be found where they vary to an extreme degree; and these have an important bearing upon the theory of septation; for cases of incomplete septation are often to be recognized in the mature sorus, while the study of earlier stages has revealed almost all imaginable steps between the single loculus and two loculi lying side by side as an obvious pair. Such pairs of loculi are common; sometimes the septum between them is of the average thickness; sometimes thin, but still complete, with firm, permanent tissue continuous across it; sometimes the permanent tissue is incomplete, and the septum composed in its middle part only of tapetum, which disappears at maturity; sometimes a large loculus will be seen with only slight encroachments of tapetum upon the sporogenous group, thus indicating the position, though not realizing the complete development of a possible septum; the last step of the series would be simply a loculus larger than the average, and these are common.

An analytical study of the tissues shows that the tissue of these partial septa may have either of two sources of origin: (1) single cells of the sporogenous group are liable to development as sterile cells; this has been seen in sporangia which even approach the normal; (2) the surrounding tissue may encroach on the sporogenous group, in the form of tapetal cells, which, when their development is considerable, is followed by cells of permanent tissue of the wall; in these cases the partial septa will be clearly seen in the mature state.

The similarity between these results and what is known in the case of septate anthers of Angiosperms is of peculiar interest; in these anthers partial septa are common, and a detailed comparison of them with those of *Danaea* suggests that the phenomena are closely alike. But, in the case of the Angiospermic anthers, we conclude, on comparative grounds, that progressive septation has taken place; this would indirectly support the view that the sorus of *Danaea* is also a result of progressive septation.

Obviously such a series of stages as that presented by *Danaea* may be read either way, and it would be possible to urge that in them we have evidence, not of progressive septation, but of fusion of loculi. This question must be considered on grounds of general probability. Without at the moment declaring a final opinion (though I think the probability is largely in favour of a view of progressive septation), this point, at least, seems clear: *that in Danaea the identity of the sporangium or loculus is not strictly defined.* To arrive at this point is,

in my opinion, a matter of some importance; the study of the sporangium in Pteridophyta has long been based upon the examination of the highly specialized and strictly constructed sporangium of the Leptosporangiatae. The conclusion is, however, becoming obvious that such strictness of construction and regularity of segmentation is exceptional, and that in the Eusporangiatae such strictness is not the rule.

Other Marattiaceae, and especially *Kaulfussia*, have also been examined, and they are all found to conform to one fundamental type, though differing in detail; it appears that, as regards the sorus, *Danaea* is the least specialized, and *Angiopteris* the most specialized, of the living genera, and that they form a very natural series. Such a series in plants of so antique a stock deserves the most careful comparative study, and the results should carry unusual weight.

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DIRECT NUCLEAR DIVISION IN THE EMBRYO-SAC OF LILIUM MARTAGON.—In the course of some work on the nuclear divisions immediately preceding the formation of the ovum in *Lilium Martagon*, I have observed the interesting fact that the two lower antipodal nuclei appear to be regularly formed by a process of direct division.

The primary nucleus of the embryo-sac divides by the indirect method, and the daughter-nuclei divide again after a very short period of rest. These divisions have been frequently figured as typical examples of karyokinesis. The four nuclei thus formed remain in the resting condition for some time, during which the embryo-sac increases greatly in length. At first the resting nuclei are all alike, in spite of the curious difference in the number of chromosomes which go to build up the micropylar and chalazal pair respectively (Guignard, Nouvelles Études sur la Fécondation, p. 187). But towards the end of this resting period the nuclei are differentiated in pairs. At the micropylar end the two nuclei have increased little in size since their formation. They are usually rather egg-shaped, and are separated from the chalazal nuclei by a vacuole which occupies the centre of the embryo-sac. The two chalazal nuclei have grown a good deal: the upper one is usually somewhat flattened, and the lower one of rather irregular outline, fitting neatly into the pointed chalazal end of the embryo-sac.

When the four nuclei show signs of approaching division, the appearance of the lowest one is very different from that of the upper three. In these the coiled chromatic ribbon which marks the spirem- (or *knäuel-*) stage is beautifully differentiated, while the lowest (chalazal) nucleus still has the reticulated appearance of the resting stage. Later on each of the micropylar nuclei has formed a small spindle and the upper chalazal nucleus a larger one, while the lower chalazal nucleus has divided in such a way that each segment appears as a hemispherical reticulated shell. These shells are connected by numerous fibrils, apparently formed by the stretching of the network in the equatorial region of the mother-nucleus. The division of the lower chalazal nucleus is complete before the three karyokinetic figures have passed out of the nuclear-plate-stage. The daughter-nuclei of course appear in the resting stage, and are connected by numerous fine threads which represent fairly enough the usual 'connecting threads' of the later karyokinetic stages. Indeed the whole appearance of this lowest pair of nuclei is now that of a rather poorly preserved di-spirem figure. The three spindles which occupy the rest of the embryo-sac are typical examples of karyokinetic figures, and form a curious contrast to the ill-defined appearance presented at the chalazal end.

I cannot do more here than allude to the possible theoretical importance of this distinction between the formation of the two lowest antipodal nuclei and that of the other six nuclei which are found in the embryo-sac before fertilization. So far as it goes, it tends to confirm Weismann's view that the elaborate mechanism of karyokinesis provides for the transmission of hereditary qualities. I hope to discuss the question at length when the details of my work are published.

ETHEL SARGANT.

On the Prothallus and Embryo of Danaea simplicifolia, Rudge.

BY

GEORGE BREBNER.

With Plate IX.

A LARGE number of young plants of *Danaea simplicifolia*, Rudge, were forwarded to the Royal Gardens, Kew, in the spring of 1895, by G. S. Jenman, Esq., F.L.S., Superintendent of the Botanic Gardens, British Guiana. At the suggestion of Dr. D. H. Scott, F.R.S, Honorary Keeper of the Jodrell Laboratory, I undertook the examination of the material.

Among the youngest plants a few prothalli were found, which showed an abundance of adult, as well as degenerating, archegonia, and also afforded sufficient material of healthy young and adult antheridia to enable their structure and development to be satisfactorily followed. Some of the archegonia contained embryos.

The fact that hitherto the prothallus and embryo of *Danaea* have not been described, makes the present opportunity rather a fortunate one, on account of the large amount of attention which is being given at present to the study of both the sporophyte and the gametophyte of the Marattiaceae.

PROTHALLUS.

In its essential characters the prothallus of *Danaea simplicifolia* agrees with that of the other genera of the

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Marattiaceae, in being more than one cell thick except at the margin, in having the archegonia distributed on a median cushion and the antheridia almost anywhere on the lower or upper surface. In general appearance the prothallus is somewhat different from that of *Angiopteris* and *Marattia* (cf. Figs. 1 and 2 with the prothalli figured by Jonkman[1], Farmer[2], and Campbell[3]).

The prothallus may, or may not, be deeply notched at the anterior growing margin (cf. Figs. 1, 2, and 3), and has evidently great capacity for long-continued growth, as in the other genera (cf. Figs. 3, 4, and 5). In some cases the prothalli attached to the young plants were nearly twice the diameter of those bearing embryos. As already indicated, the archegonia usually occur on a median cushion, the limits of which are not sharply defined. In one instance a small cushion, with a few archegonia, was found on one of the heart-shaped lobes quite remote from the median line and apical margin. This agrees with Prof. Campbell's statement, that[4], in the Marattiaceae, 'the sexual organs of the lobes are *mainly* (the italics are mine) antheridia.'

The antheridia are truly ubiquitous, occurring freely on the under and upper surfaces of the main part of the prothallus, on the lobes, and also, but more sparingly, on the median cushion amongst the archegonia. Marginal cushions are not uncommon; they are literally studded with antheridia in such a manner that some of the antheridia open on the edge of the prothallus. The occurrence of antheridia on the lateral portions of the prothallus agrees with Luerssen's observations on *Angiopteris*[5], which Jonkman was not able

[1] Jonkmann, H. F., La génération sexuée des Marattiacées. Archives Néerlandaises, T. xv. p. 199.

[2] Farmer, J. B., On the Embryogeny of *Angiopteris evecta, Hoffm.* Annals of Botany, Vol. vi. No. XXIII. October, 1892.

[3] Campbell, D. H., (a) Observations on the development of *Marattia Douglasii*, Baker. Annals of Botany, Vol. viii. No. XXIX, March, 1894; (b) The Structure and Development of the Mosses and Ferns. Macmillan and Co., 1895, p. 254 et seq.

[4] Loc. cit. (b), p. 258.

[5] Luerssen, Ueber die Entwickelungsgeschichte des Marattiaceenvorkeims. Bot. Zeit. 1775; Medicinisch-pharmaceutische Botanik, Bd. I. p. 581.

to confirm in the prothalli examined by him[1]; neither observer, however, found them on the actual margin. Even after the cotyledon of the young plant has completed its development, antheridia may still be found in process of formation on the prothallus (cf. Fig. 3 at *an.*).

Perhaps the most notable point about the prothallus of *D. simplicifolia* is that the rhizoids are septate, three or four transverse walls being readily observable (cf. Fig. 6). Prof. Campbell[2] mentions, with regard to the other genera of the Marattiaceae, that the 'root-hairs' of the prothallus are unicellular[3]. These rhizoids of *Danaea* remind one not a little of the protonema of a Moss.

ANTHERIDIUM.

The antheridia agree in every respect with those of *Angiopteris* and *Marattia*, at least with those which may be considered as of the normal type (cf. Fig. 12 with the corresponding figures of Jonkman and Campbell's papers). The figures for one genus would do almost as well for either of the other two. A modification in the usual mode of development, which has not been described for either *Angiopteris* or *Marattia*, was, however, noticed in *D. simplicifolia*, although there is no reason why it should not occur in these genera also. The usual process, to quote Prof. Campbell[4], is as follows:—'The antheridium arises from a single superficial cell, which first divides into an inner cell, the mother-cell of the sperm-cells, and an outer cover-cell. The latter divides by several curved vertical walls which intersect, and the last wall cuts off a small triangular cell, which is thrown off when the antheridium opens and allows the sperm-cells to escape. Before these are completed, however, cells are cut off from the adjacent cells of the prothallium, completely enclosing the mass of sperm-cells.' The peculiarity observed in *Danaea*

[1] Loc. cit., p. 211. [2] Loc. cit. (b), p. 257.

[3] I was able to confirm this statement with regard to *Angiopteris*, Prof. J. B. Farmer having very kindly placed prothalli bearing young plants at my disposal.

[4] Loc. cit. (a), p. 5.

is that not infrequently the formation of the curved intersecting vertical walls, even down to the last, may precede the horizontal wall, which cuts off the mother-cell of the sperm-cells (cf. Figs. 7, 9, and 10). All these figures were taken from antheridia developing on the prothallus, in which the mother-cells involved had already reached, or nearly reached, their adult condition before commencing to form the antheridia. In the case of the two latter figures, a developing antheridium of this type is represented, as seen from below (Fig. 9) and from above (Fig. 10). As far as the drawings permitted, corresponding walls have been lettered alike; but to realize the point clearly it might be as well to make a tracing of Fig. 10, and then turn it over and superpose it on Fig. 9, when the relation of the walls would at once become obvious. In Fig. 9 the two cells which will ultimately give rise to the spermatozoids are still quite young and merismatic, whereas in Fig. 10 the cover-cells have already reached their adult condition. This condition differs considerably from that represented by Prof. Campbell's Figs. 8, 9, and 10[1], where the growth of the antheridium as a whole goes on *pari passu* with that of the prothallus. As a result of the exceptional mode of development, in the most extreme case—and such was actually observed—there may be only a single vertical row of four spermatozoid-mother-cells visible in one plane, whereas in a normal case (e.g. Fig. 12) five vertical rows, each of four spermatozoid-mother-cells, may occur. Jonkman noted the fact that the number of spermatozoids formed in *Angiopteris* and *Marattia* was very variable, and gave as observed numbers 20–500 or even more[2].—This exceptional state of matters in *Danaea* can only be looked upon as a further reduction due to late development.

Archegonium.

Material was not available for following the development of the archegonia, but the adult structure is essentially the

[1] Loc. cit. (a), Pl. I.

[2] Loc. cit., p. 214.

same as that of *Angiopteris*, as figured by Jonkman and Farmer[1], as also of *Marattia Douglasii*, Baker, figured by Campbell[2]. Jonkman pointed out the fact that in *Angiopteris* the number of neck-cells in two of the vertical rows is frequently three, while in the other two it is four, sometimes four and five, rarely two and three respectively[3]. The last of these three conditions occurs most frequently in the case of *Danaea simplicifolia*, in which there are only two cells in two of the vertical rows and three in the other two. Jonkman likewise noted the variability in the formation of the accessory cells (*m.*), which are cut off from the cells adjacent to the developing archegonia. In *D. simplicifolia* this variability seems to be carried further, so that comparatively rarely were archegonia seen with the full complement of these cells. The corresponding cells of the antheridium (cf. Figs. 9 and 12, *m.*) are much more constant, a fact which is readily understood, since they are doubtless functional in aiding the discharge of the spermatozoids at maturity. In Fig. 13 the structure marked *sp.* is in all probability a spermatozoid, *ov.* is the ovum. In Fig. 14 the condition of the neck, already referred to, is quite obvious, there being only two neck-cells on one side and three on the other. In the case of Fig. 13 it was seen that the two rows behind, which could not be figured, consisted each of three neck-cells[4].

Embryo.

Embryos in the earlier stages of development were not found, the youngest being represented in Fig. 15. It was obtained from the prothallus of Fig. 2, and forms a good sequel to Prof. Campbell's Fig. 22[5]. Most of the divisions of the latter are still fairly readily recognizable in the former. This figure was taken from a hand-section, and its exact

[1] Loc. cit., Fig. 1. [2] Loc. cit. (a), Fig. 16. [3] Loc. cit., p. 216.

[4] For theoretical considerations concerning the affinities of the Marattiaceous archegonium, see Campbell, loc. cit. (a), p. 7.

[5] Loc. cit. (a).

position could not be determined, although there is every reason to believe that it was not far off the median line[1].

Prof. Farmer[2], and likewise Prof. Campbell, states that it is the anterior epibasal octants which give rise to the cotyledon. The embryos of Figs. 16 and 18 agree with their observations in this respect, as they were in both cases presumably developed near the growing apex of the prothallus, at least for the time being, the cotyledon being anterior. With regard to the embryo of Fig. 16, it was impossible to say which was the original axis of growth, as the development of the prothallus had been exceedingly erratic. The embryo of Fig. 18 was from the prothallus of Fig. 1, in which its position is indicated by the black dot. With regard to Fig. 15 from the prothallus of Fig. 2, the direction of the growing margin is indicated by the arrow; but as this section is not median, it is impossible to decide which epibasal octants are giving rise respectively to stem and to cotyledon. With a view to try and settle this point for *Danaea simplicifolia*, as embryos were not available, a young plant which had only expanded its first leaf, or cotyledon, was microtomed along an axis at right-angles to the growing edge, as there was some doubt about the attached prothallus being entire; in fact, it was probably only a large lobe with an adventitious cushion. Be that as it may, this series showed without a doubt that the cotyledon must have been developed from the *posterior epibasal* octants, and the stem-apex from the anterior, unless there had been subsequent rotation. This seems to indicate that in *Danaea*, at least, the orientation of the embryo is not constant. Seeing that the embryo penetrates vertically through the prothallus, any definite orientation as regards its epibasal octants becomes unimportant, so long as they are turned towards the upper side. This consideration gives the clue to the inconstancy observed[3].

[1] Careful re-examination of the sister-section, which is rather thick, has shown that it contains the apical cell (or cells ?) of the stem and cotyledon, and that in it the line II . . . II divides the embryo more nearly into two equal halves.

[2] Loc. cit., p. 267.

[3] This question cannot be considered as really settled till a far greater number

COTYLEDON.

The adult cotyledon is a very simple spathulate leaf without stipules[1] (cf. Figs. 3 and 4), and the venation is monopodial in its branching, thus agreeing with *Angiopteris*[2] and differing from *Marattia Douglasii*[3].

The vascular bundle is very simple, consisting sometimes of only two or three tracheids and a small amount of phloem. It may be considered collateral, but this depends on whether the layer of parenchymatous cells towards the upper surface is to be looked upon as phloem or otherwise. Certain it is that phloem, with recognizable sieve-tubes, occurs only on the underside and flanks of the bundle.

THE STEM.

The primary stem, which is comparatively short, has a small concentric bundle, or stele, of the ordinary Marattiaceous type, and is somewhat elongated in the same plane as the diarch xylem-plate of the root, the stele of which merges so gradually into that of the stem that it is impossible to determine where the one ends and the other begins. With regard to the phloem, the transition first shows itself by a gradual increase in the number of the thin-walled elements at the poles of the xylem-plate of the root, so that the protoxylem no longer abuts directly on the endodermis, or is separated from it by

of embryos have been examined under conditions more favourable for determining their orientation.

[1] *Danaea* agrees with the other genera of the Marattiaceae in having well-developed stipules to the older fronds, and, moreover, the sclerenchyma of the petiole, &c. is of the *usual* Marattiaceous type. Holle in his paper '*Ueber die Vegetationsorgane der Marattiaceen* (Sitzung der Königlichen Gesellschaft der Wissenschaften zu Göttingen, Jan. 8, 1876), made a mistake in these two respects, which was subsequently corrected by Kühn (*Ueber den anatomischen Bau von Danaea*, Flora, 1890), who pointed out that *Danaea* differs anatomically in *no* essential respect from the rest of the Marattiaceae. There is little doubt, as Kühn suggests, that Holle had incorrectly-named specimens to deal with. I myself have had the opportunity of verifying Kühn's statements in several species of *Danaea*.

[2] Farmer, loc. cit., Fig. 19.

[3] Campbell, loc. cit. (a), p. 11 and Fig. 27.

only one thin-walled element. Passing upwards, the thin-walled tissue in question increases in amount till it becomes recognizable as part of the phloem of the primary stem. With regard to the xylem, the details of the junction of the stele of the root with that of the stem could not be made out with anything like certainty; this was also the case with regard to the transition from the xylem of the stem to that of the cotyledonary trace. All that can be safely said is that in both cases the elements in the transitional region seem to be exclusively scalariform tracheids[1]. The stele of the primary stem has a well-marked endodermis (Fig. 20, *en.*), and in this *Danaea* agrees with *Angiopteris*[2], but differs in not having a central mass of parenchymatous tissue. Fig. 20 shows the stele just about to divide to form the traces of the cotyledon and of the second leaf. In some cases, as already mentioned, the xylem of the cotyledon consists of only two elements, arranged side by side in the tangential plane. In such a case it is obviously impossible to speak of a protoxylem as distinguished from a later formed xylem. In cotyledons with more vigorous bundles, protoxylem is readily enough recognizable on the inner (axial) side; it is not, however, composed of spiral elements, but of delicate tracheids of narrow calibre with wide scalariform pits. The arrangement of the steles in the stem of young and older plants need not be discussed here[3], but the mode of succession of the first three leaves is exactly the same as in *Angiopteris*, as described by Prof. Farmer[4], and thus the first part of the spiral is laid down; the anatomy, however, becomes subsequently much complicated by anastomoses of the steles. The adventitious roots seem to arise much later than in *Angiopteris*, in which the *second* leaf already has its accompanying adventitious root

[1] The series of microtome-sections obtained through the parts in question were not very successful, and the young plants were not at the best stage for the purpose.

[2] Leclere du Sablon, *Recherches sur la tige des fougères.* Ann. des Sciences naturelles, 1890, T. ii.

[3] This question will be more fully treated in a paper on the comparative anatomy of the Marattiaceae, which is in process of preparation.

[4] Loc. cit., p. 270.

which emerges just beneath the cotyledon[1]. In plants of *Danaea simplicifolia*, in which several leaves had already expanded, there was no external evidence of adventitious roots. These, when they do appear, come out through the bases of the leaves next below those to which they belong, and for a long time, at any rate, there is only one root to each leaf.

The ground tissue of the primary stem is composed of ordinary parenchyma being fairly bulky. Tannin-sacs[2] of the ordinary Marattiaceous type early make their appearance, but the mucilage-canals do not do so till comparatively late, there being no sign of them in the first few leaves at any rate.

The Root.

The primary root has a simple diarch stele, the xylem-plate lying in approximately the same plane as the bundles of the first two leaves. The phloem occupies the usual position, and is of the kind characteristic of the group. *Danaea* agrees with *Angiopteris*[3] in having a diarch xylem-plate, but differs from *Marattia Douglasii*, which has usually a tetrarch stele[4]. There is a well-marked endodermis, and again in this respect *Danaea simplicifolia* agrees with *Angiopteris* and differs from *M. Douglasii*[5].

The primary root emerges from the prothallus long before the cotyledon (cf. Fig. 8). The embryo of this figure was, it is true, the only one sufficiently advanced to show this; but there is no reason to regard this as other than the normal case. Moreover, two of the embryos figured by Prof. Farmer show a tendency in the same direction[6]. This seems to completely nullify the importance attached to the converse condition, found in *M. Douglasii*, by Prof. Campbell[7].

It is rather interesting to note that the root-hairs of the primary root are of exactly the same type as the rhizoids of

[1] Farmer, loc. cit., p. 270.
[2] Farmer, loc. cit., p. 269.
[3] Leclere du Sablon, loc. cit.
[4] Campbell, loc. cit. (a), p. 14.
[5] Loc. cit. (a), p. 14.
[6] Loc. cit., Figs. 9 and 10.
[7] Loc. cit., p. 14.

the prothallus, viz. long narrow hairs with a few transverse walls, the component cells being distinctly uni-nucleate. The figure of the rhizoid of the prothallus would do just as well for a root-hair of the young sporophyte.

Apical Growth.

The vexed question of the apical growth in stem, root, and cotyledon is not much cleared up by the stages secured in *D. simplicifolia*. The stem of the embryo, represented in Fig. 16, seems to have a well-marked single apical cell at *st*. This is represented on a larger scale in Fig. 17. There is probably a certain amount of obliquity in this section, as the young bundle is not seen passing into the cotyledon at this point, but in the fourth preceding section of the series. This much is certain, however, that the cell in question is absolutely the largest anywhere near the apex of the stem, and likewise possesses the largest nucleus. On account of its size, and of its position in relation to the cotyledon, it is practically certain that it is the apical cell. With regard to the embryo of Fig. 18, there is probably still a single apical cell in the growing-point of the stem, but rapidly approaching the time when it will be merged in a group of equivalent initials. It is already hardly, if at all, larger in size than one or two of the adjacent cells, but its nucleus is undoubtedly the largest and at the same time richest in chromatin (cf. Fig. 19); hence, in all probability, it is the apical cell. In a series of transverse sections of a plantlet, in which only the cotyledon had expanded, and of which the second leaf was still quite young, the stem had a single apical cell with a four-sided base. The cotyledon of this plantlet was the one already referred to as having a very feebly developed bundle, the xylem consisting of only two elements. In somewhat more advanced plantlets it was quite impossible to fix on any particular cell as the apical cell, and in these cases there would be little doubt that the apical meristem consisted of a small number of equivalent initials.

With regard to the cotyledon, the somewhat late stages

available showed no definite single apical cell, and Prof. Campbell found the same to be the case with *Marattia Douglasii*[1]. A plantlet was investigated in which, besides the cotyledon and a quite young second leaf, there was the earliest rudiment of a third; but this rudiment seemed already to have a two-celled apex, one of the cells being in the last stage of karyokinesis, although the new anticlinal wall had not been formed. Hence it is exceedingly probable that in the case of the cotyledon and the subsequent leaves, if at any time there is a single apical cell it can only be of exceedingly transitory duration.

The root presented the same difficulty; the only promising young embryo, Fig. 16, did not show any cell, or cells, which could be recognized as constituting the growing-point of the future root. The root of Fig. 8 might have shown the condition at a later stage, but it unfortunately got broken off before imbedding and was sectioned by hand. It does not show anything decisive. Attempts were made to study the primary roots of young plants, but these were nearly all either actually injured, or in such bad condition at the tip as to make otherwise successful microtome-sections useless. In one case, however, a longitudinal section (one of a microtome-series) was obtained which showed a remarkably large cell which, judging from its position and the condition of the adjacent tissues, seemed to be the apical cell, although it was not quite axially median. Sections of the adventitious roots left no doubt that, in their case at least, the growing-point consisted of a few equivalent initials, in one case a well-marked group of four.

SUMMARY.

The genus *Danaea*, as exemplified by *Danaea simplicifolia*, Rudge, is found to agree very closely with *Angiopteris* and *Marattia*, both in the gametophyte and in the embryo-sporophyte. Perhaps the most important point of difference is the presence of septate, or multicellular, rhizoids in the former

[1] Loc. cit., p. 11.

genus, whereas they are unicellular in the two latter genera. It is possible this may be found to have phylogenetic importance, and strengthen the view that the Eusporangiate Ferns and the Mosses had a not very remote common ancestor.

The exceptional mode of development of the antheridia, in certain cases, resulting in the formation of a very small number of spermatozoids, is probably due to the antheridium as a whole being developed from a cell which was nearly, or quite, adult.

The archegonia present no features which are not likewise characteristic of *Angiopteris*, *Marattia*, and presumably *Kaulfussia*.

The structure of the growing-point of the stem of *Danaea* seems to be very much in the same condition as in the other two genera which have been studied. It appears in fact to be still in the transition stage from a single apical cell to a group of equivalent initials. It is interesting to note that *Danaea*, which is usually placed lowest in the scale among the Marattiaceae, is the genus which presents the clearest indications of a single apical cell. There can be little doubt, in view of the results obtained, that the Marattiaceae are descended from an ancestor whose growing-point presented a well-marked single apical cell, that being really the primitive type of growing-point throughout for the great Fern-series.

The apical meristem of the cotyledon and subsequent leaves does not seem at any time to obviously possess an apical cell, and in this also there is agreement with the other genera.

The primary root, until further evidence is forthcoming, may be considered to have a single apical cell; but the subsequent adventitious roots have a group of equivalent initials, sometimes clearly four.

The stele, both of the stem, and of the root, has a distinct endodermis, agreeing in this with *Angiopteris* at any rate. The root-stele merges directly in that of the stem, the latter

(i. e. stele of the stem) of course consisting of the leaf-trace-bundle of the cotyledon until complications arise through the addition of the subsequent leaf-traces. The junction is effected by ordinary scalariform tracheids.

Note.—In No. 15 of the Botanisches Centralblatt for 1896 (Band LXVI, p. 49), Jonkman has a preliminary communication on the embryogeny of *Angiopteris* and *Marattia*, the special interest of which lies in the fact that the embryos were obtained by cultivation. He, so far, does little more than confirm previous observations; the most important point, however, being that he finds that the growing-point of the embryonic stem consists of a few initials, and that of the root of four. This agrees in the main with the results of previous observers; but Jonkman is disposed to consider these conditions as constant, whereas Prof. Campbell and, in the present paper, myself are of the opinion that there is a certain amount of inconstancy, and that occasionally, if not frequently, the embryonic stem, as also the root, has a single apical cell. It is somewhat interesting that Jonkman finds in *Angiopteris* and *Marattia* a group of four equivalent initials in the growing-point of the primary root, a number which was found in the young adventitious roots of *Danaea simplicifolia*, although the condition of the primary root was not satisfactorily made out in the latter. Prof. Farmer had come to the conclusion, with regard to *Angiopteris evecta*, Hoffm., that in the embryonic root there is a single apical cell, which has a very transitory existence as such.

EXPLANATION OF FIGURES IN PLATE IX.

Illustrating Mr. Brebner's paper on the prothallus and embryo of *Danaea simplicifolia*, Rudge.

Figs. 1 and 2. Prothalli. Nat. size.

Fig. 3. Young plant attached to prothallus, with only the cotyledon expanded: *an.*, developing antheridia. × 2½.

Fig. 4. Similar to preceding: *pr.*, prothallus. Nat. size.

Fig. 5. Young plant still attached to prothallus, *pr.*, showing three young leaves and the bases of the two first. Nat. size.

Fig. 6. Rhizoid, *rh.*, of prothallus, *pr.* × 50.

Fig. 7. Young antheridium originating from adult cell of prothallus. × 350.

Fig. 8. Young antheridium, slightly older than the preceding, and showing the first division of the mother-cell of the sperm-cells. × 350.

Fig. 9. Young antheridium, similar to preceding, seen from below: *m.*, peripheral cells. × 350.

Fig. 10. Cover-cells of preceding, seen from above. × 350.

Fig. 11. Cover-cells of empty antheridia, seen from above. The dotted lines in one of the sets indicate the thickness of the cells seen in perspective. × 350.

Fig. 12. Nearly mature antheridium: *m.*, peripheral cells. × 350.

Fig. 13. Mature archegonium: *ov.*, ovum; *sp.*, probably a spermatozoid. × 350.

Fig. 14. Mature archegonium: *ov.*, ovum; *n.c.*, neck-cells. × 350.

Fig. 15. Vertical section of a youngish embryo, not quite median: *b.b.* basal wall; *II. II.*, transversal wall. Arrow indicates direction of growing-point of prothallus. × 250.

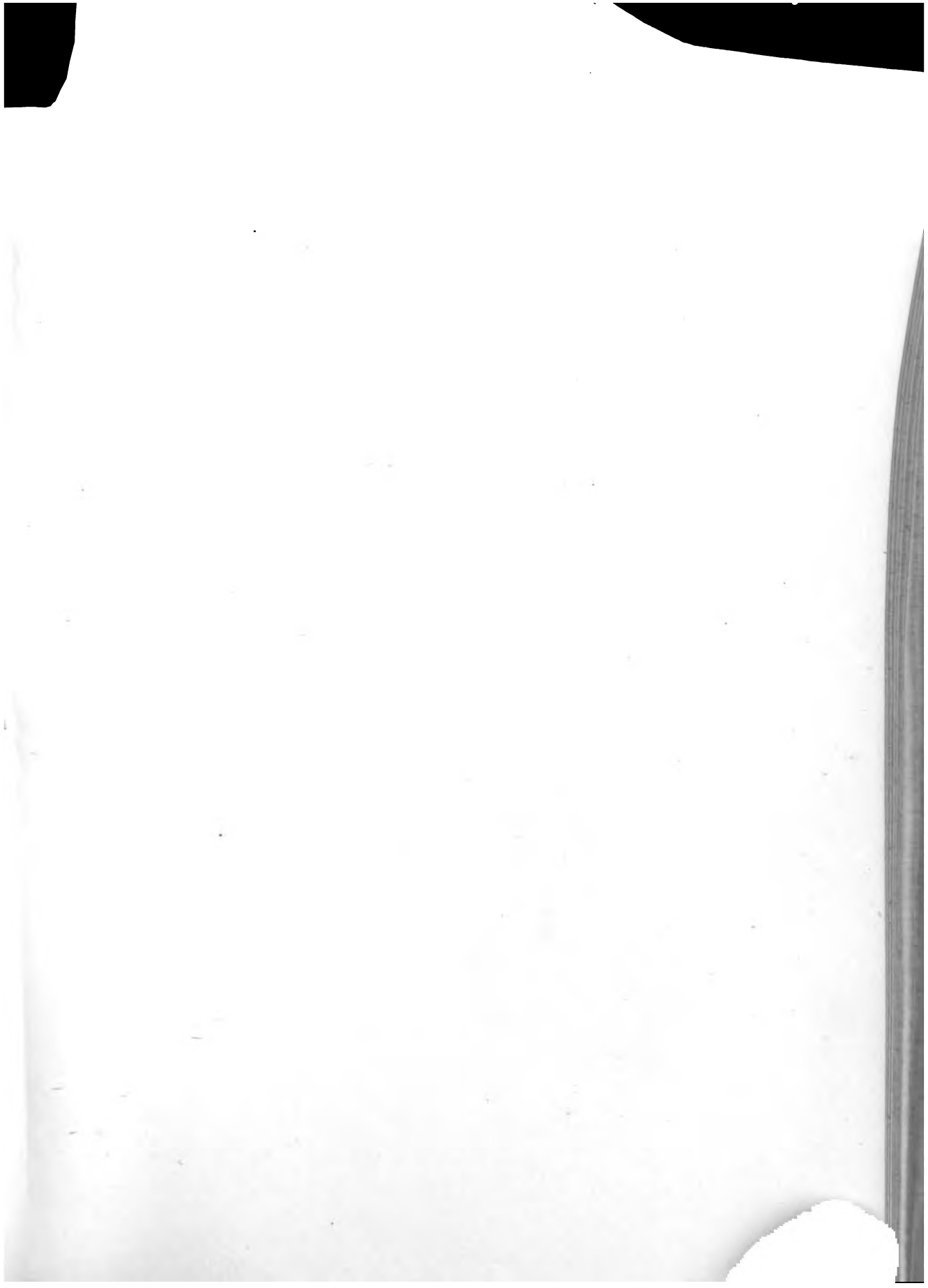
Fig. 16. Vertical section of an older embryo: *st.*, growing-point of stem; *L.*, cotyledon. × 200.

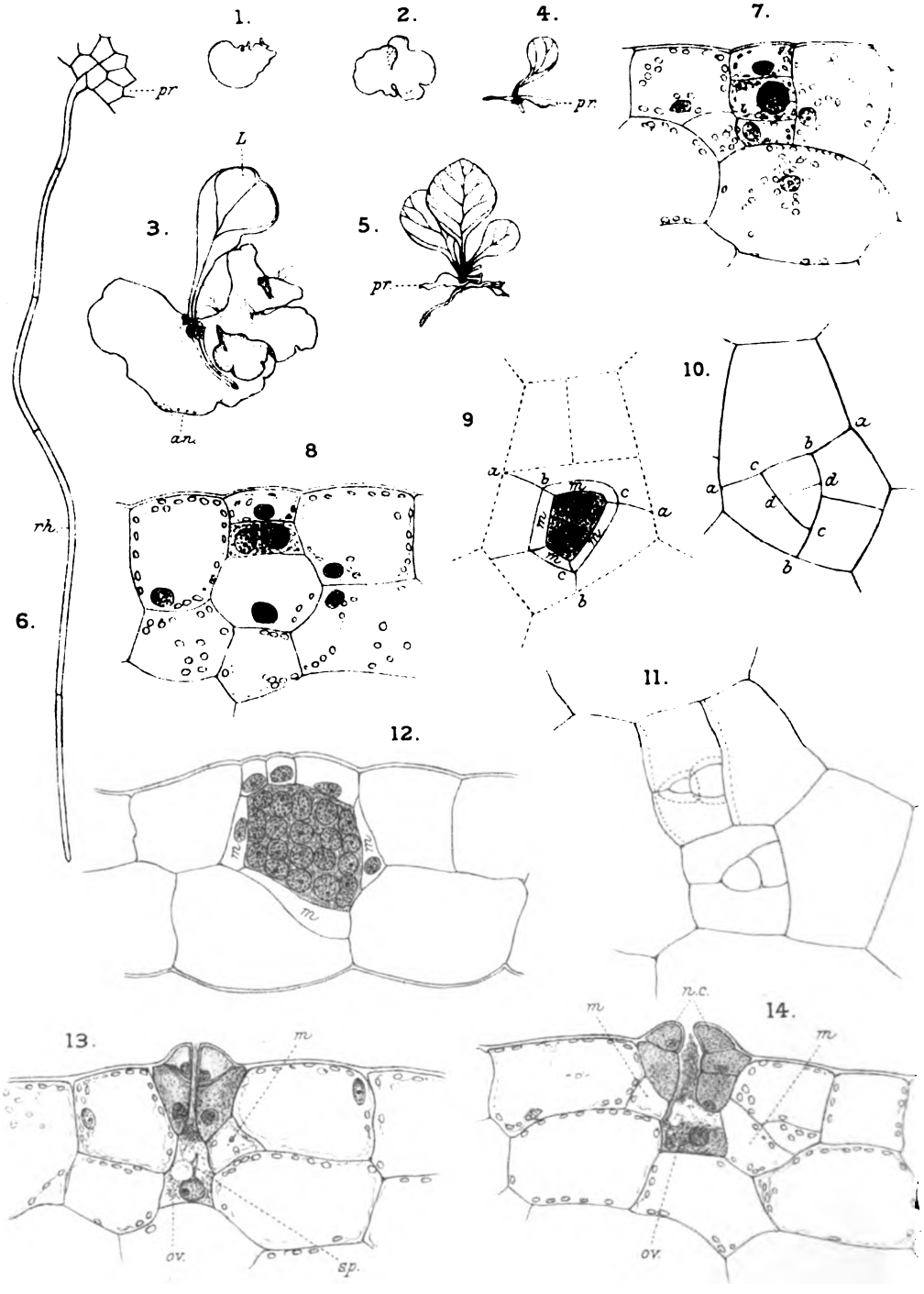
Fig. 17. Growing-point of stem of the same. × 400.

Fig. 18. Vertical section of a still older embryo: *st.*, growing-point of stem; *L.*, cotyledon; *r.* root. × 100.

Fig. 19. Growing-point of stem of the same. × 400.

Fig. 20. Transverse section of stele of the stem at junction of first and second leaf traces: *L. b.*, cotyledon bundle; *en.* endodermis. × 400.

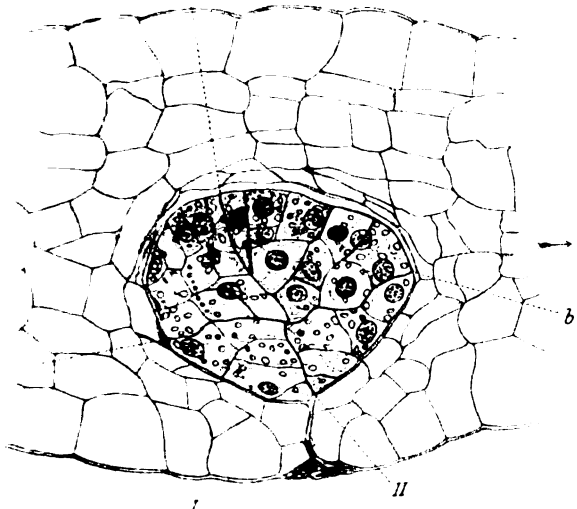




G. Brebner del.

BREBNER. — DANAEA.

15.



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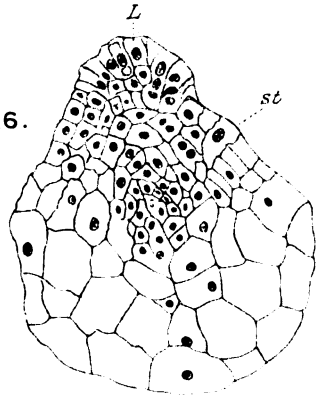

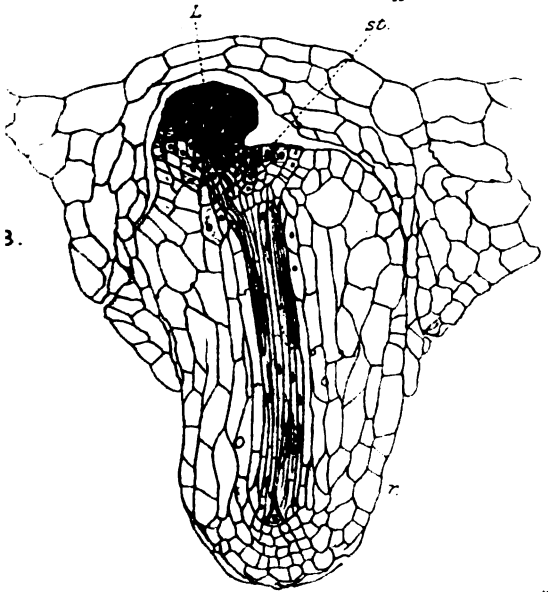

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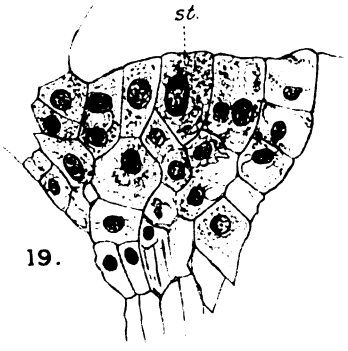

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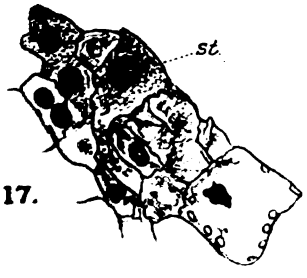

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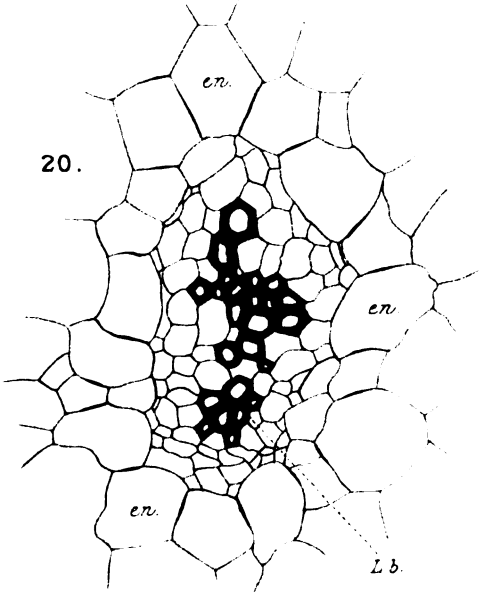

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A Revision of the Genus Coprinus.

BY

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With Plates X and XI.

THE Fungi constituting the genus *Coprinus*, as at present understood, were originally included under the collective name of *Agaricus*, which at one time covered the whole of the gill-bearing Agarics. Persoon[1] was the first to detect, in the complete deliquescence of the gills at maturity, a feature of sufficient importance to justify the creation of a special section of *Agaricus*, under the name of *Coprinus*, for the reception of those species possessing this peculiarity. At a later date Fries[2] raised Persoon's section to generic rank, retaining the name *Coprinus*, characterized mainly by the feature indicated by Persoon, the solution of the gills into a dripping, inky liquid at maturity. In reality, this one biological character is the only constant point of distinction between *Coprinus* and the remainder of the fleshy, putrescent Agaricineae. Morphologically there is only a relative difference; the statement by Fries in his definition of the genus *Coprinus*, that the trama is obsolete, is not correct; a well-developed trama is always present, formed of interlaced septate hyphae, often furnished with vesicular swellings, running parallel with the sides of the gill or lamella, and

[1] Syst. Meth. Fung., p. 395 (1831).
[2] Epicr. Syst. Mycol., p. 241 (1836–1838).

[Annals of Botany, Vol. X. No. XXXVIII. June, 1896.]

giving off free ends which bend outwards, and bear the elements of the hymenium, exactly as in other Agarics. Cystidia are present in the hymenium of most species, and are, as a rule, much larger than elsewhere in the Agaricineae. The spores also, as a rule, are relatively large, one Queensland species—*Coprinus gigasporous*, Massee—having the largest spores of any known Agaric, measuring 28–30 × 14–16 μ. In *Coprinus insignis*, Peck, the spores are minutely asperate; in all other known species the epispore is smooth. In systematic works the spores are said to be black; that is, when seen in the mass on a white ground; and under these conditions the statement is approximately correct, being often accompanied by a tinge of purple or brown. When seen by transmitted light the spores are always some shade of brown, varying in the different species from rich burnt-sienna, through umber, to apparent black, when the colouring matter is so dense that the spore is quite opaque. Karsten[1] has recently broken up the genus *Coprinus* into several genera, depending mainly on the shade of colour of the spores, as seen by transmitted light; but, like all attempts at classification based on a single character, whatever may be its merits when treating of the species of a limited area, it breaks down when dealing with the entire number of known species; and even in a local flora, the adoption of narrow characters that will not embrace all known species is perhaps a mistake, inasmuch as it cramps the student's knowledge, and leads him to believe that genera and species are much more sharply defined than they are in reality.

Coprinus sclerotigenus, Ellis and Everh., springs singly or in small numbers from a large, irregular, externally black sclerotium. *C. tuberosus*, Quélet, also originates from a small, black sclerotium; and Brefeld[2] has shown that a small sclerotium is formed by *C. stercorarius*, Fr. The number of sclerotium-forming species will probably soon be extended, now that attention has been directed to the subject.

[1] Ryssl. Finl. och den Skandinaviska Hattsvampar, I. pp. 526–550 (1879).
[2] Bot. Untersuch. über Schimmelpilze, Heft III (1887).

The flesh of the pileus is very thin in all the species of *Coprinus*, and in many kinds is reduced to such a delicate membrane that Fries considered it to be entirely wanting, and in his latest work[1] divides the genus into two tribes, founded respectively on the supposed presence or absence of a cuticle or layer of flesh covering the gills. The species included in the first tribe—Pelliculosi—have a pellicle, and when the pileus becomes upturned and split at maturity, the splitting takes place through the pellicle, between the gills. In the second tribe—Veliformes—the pellicle is supposed by Fries to be absent, but this view will be shown to be a mistaken one; the radiating grooves which appear as the pileus expands are due to the splitting of the gills themselves, commencing at the back and continuing towards the free edge of each gill, owing to the trama offering least resistance, and consequently giving way first during the expansion of the pileus. The hyphae of the trama do not deliquesce during the splitting of the gill, but are torn apart. As already stated, a very thin but continuous layer of flesh is present, covering the surface of the pileus in the members of the tribe Veliformes; the portion nearest the gills consisting of slender septate interwoven hyphae, the free ends of which give origin to a layer of piriform or subglobose cells, closely packed side by side like the palisade-tissue of a leaf, and forming the free surface of the pileus. During expansion the large cells forming the surface of the pileus are torn apart, but persist in a dry condition, and produce the scurfy or furfuraceous appearance presented by the pileus of all the species of the tribe Veliformes during expansion. Fig. 25 is a section through a portion of the pileus of *Coprinus plicatilis*, Fries, and shows the splitting of the gills, also the scurfy appearance of the pileus, due to the separated large external cells of the pellicle.

There is no trace of a secondary veil present in any known species of *Coprinus*: hence a true ring or annulus is never

[1] Hym. Eur., p. 320 (1874).

present on the stem, the structure called the ring in systematic works being, so far as *Coprinus* is concerned, the free margin of the volva, or basal portion of the primary or universal veil, which, in some of the more highly organized species, breaks away from the lower fixed portion of the volva, and is carried for some distance up the stem during the increase in length of the latter. In some species, as *Coprinus Hendersoni*, Berk., this structure is situated about half-way up the stem, to which it is attached by its lower margin, and consequently looks much like a true ring formed from the remains of the secondary veil; but it is in reality only the free margin of the volva, the lower portion of which closely embraces the stem, and is of a looser texture than that of the true stem above the margin of the volva. When the stem is thus surrounded for a considerable portion of its length by an adnate volva, it is said to be peronate. This appearance is produced by the increase in length of the stem being due mostly to basal growth, and in the volva growing at the same rate as the stem it closely surrounds; when the volva does not grow at the same rate as the stem, then its free margin only is carried up by the elongating stem, as frequently seen in *Coprinus comatus*, Fries. This explanation is equally applicable to all Agarics with a peronate stem.

A clear idea of the broad lines of evolution—morphological and physiological—presented by the Agaricineae is necessary to enable the reader to judge of the view put forward respecting the genetic relationship of the genus *Coprinus* with the remainder of the series.

Morphologically, the lowest and most primitive type of structure met with in the Agaricineae is illustrated by species included in such genera as *Marasmius*, *Pleurotus*, and *Claudopus*, where the pileus is sessile or stemless, and fixed by its back to the substratum, the gills being uppermost and consequently entirely unprotected from the earliest stage of development. In the second type the pileus is furnished with a more or less evident lateral stem, formed by the outgrowth of a point of the margin of the pileus, the gills

radiating from the stem-like point; such Fungi grow horizontally, and in the most perfect representatives the pileus has reversed the position characteristic of the first group, and we find the gills occupying the under surface, pointing towards the ground. *Panus stypticus* illustrates this stage of development. In type three, illustrated by the species of *Paxillus*, we get shadowed in the umbrella-type of structure, so characteristic of the Agaricineae; the stem has crept within the margin of the pileus, at first near to one edge, or excentric; in the higher species originating from the centre of the under surface, or central; the gills always run down the stem for some distance, thinning out into mere lines, and are described as decurrent. In type four, which includes the greatest number of species, the stem is always central, the gills never decurrent, springing from the stem by their entire width—adnate—or more or less cut out behind—adnexed—but always touching the stem. In the fifth and highest type of structure, the stem is central, and the gills are so much cut out behind that they are quite free from the stem, as in the genera *Pluteus*, *Amanita*, &c.

From the above it will be seen that there are three leading lines of departure from the primitive type of structure:—(1) Turning the hymenium downwards, away from the light, thus securing protection from sun, rain, &c., until the spores are mature: (2) The acquisition of a central stem; the stem in itself is an advantage in elevating the pileus from the ground, and thus facilitating the dispersion of the spores by wind, &c.; and its central position renders possible the closely-set radial arrangement of the gills, a plan which secures the greatest possible hymenial or spore-bearing surface with the least expenditure of material: (3) The freedom of the gills from the stem; the advantage of this point is not obvious, at least not to me, but the persistence with which it is carried out in all the highest forms prove it to be of some decided advantage.

Contemporaneously with the above phases of development we find the gradual evolution of the protective structures,

known respectively as the primary or universal veil, and the secondary veil, which are most perfect in the highest stage, and entirely absent from the lowest.

The Agaricineae do not form a single group having the sequence indicated, but are in reality broken up into four groups or series, each running through the five types of structure already explained. These four groups are characterized by the colour of the spores, as follows: spores black—Melanosporae; spores brown—Ochrosporae; spores pink or salmon-colour—Rhodosporae; and spores white—Leucospore. These four colour-groups form a sequence of development in time, the Melanosporae being the oldest, and the Leucosporae the newest. The evidence in support of this statement is as follows. In the oldest group, omitting *Coprinus*, which it has hitherto included, the five types of structure are not represented at the present day, types 1 and 2 being obsolete; the species are few in number and of world-wide distribution; the sporophore is very short-lived, and entirely lacks the differentiation of structure present in the tissues of the higher groups; the primary and secondary veils, when present, are comparatively rudimentary, and the spores are relatively large.

Passing to the newest group in time, the Leucosporae, we find the species more numerous than those of the three older groups added together; and although representatives are met with everywhere, the different sections and genera are highly characteristic of special regions. All the five types of structure have representatives, the simplest types having fewest species; the sporophore in the highest forms is fleshy, and persists for several days, producing spores in succession, thus extending the period of spore-production, and in proportion the extension of the species in space. In some genera the sporophore is corky and persistent, and from analogy with what occurs in other groups of Fungi, as the Polyporeae, may become perennial. The primary and secondary veils are highly developed, and afford complete protection to the hymenium until the spores are mature.

In certain genera, as *Hygrophorus* and *Russula*, we meet with highly differentiated groups or bundles of laticiferous hyphae, containing hyaline or coloured latex, which in some species is sapid, in others intensely hot and acrid. The by-products of metabolism are utilized in the formation of brilliant colours, and bitter or acrid and often poisonous products. The spores as a rule are minute, and thus readily dispersed by wind. The features thus indicated as characteristic of the Leucosporae, appear in a less perfect and pronounced condition in the older groups, Rhodosporae and Ochrosporae.

Finally, there is one feature of primary importance common to the whole of the Agaricineae, excepting the genus *Coprinus*; namely, the dissemination of the spores by wind, and for the purpose of effecting this object the gills are persistent—not deliquescent at maturity—and the mature spores are liberated as a very fine, dry powder.

Affinities.

In the latest scheme of classification, propounded by Saccardo [1], *Coprinus* occupies a central place in the Melanosporae. This location, however, is exceedingly unsatisfactory, owing to the much higher standard of the features characterizing *Coprinus*, as compared with those of the genera with which it is associated. A truer estimate of *Coprinus* was shown by Fries [2], who placed it on a level with the genus *Agaricus*, as interpreted by him.

As before noted, the species of *Coprinus* differ from the remainder of the Agaricineae in one important biological feature,—the deliquescence of the gills at maturity into a liquid which drips to the ground, carrying the mature spores along with it. This primitive and relatively imperfect mode of spore-dissemination, as compared with the minute, dry, wind-borne spores of the remainder of the Agaricineae, combined with other evidence to be noted later on, indicates

[1] Sylloge Fungorum, Vol. v, p. 1078 (1887).
[2] Hymenomy. Eur., p. 320 (1874).

that in the genus *Coprinus* we have, in reality, the remnant of a primitive group of Fungi, from which have descended the entire modern group of Agaricineae having wind-borne spores; and which, on the other hand, can be traced back to the still more primitive, subterranean Fungi which are the common ancestors of the entire group of the Basidiomycetes.

Evidences of the antiquity of *Coprinus* are seen in the world-wide distribution of the genus, and the limited area occupied by species; each large mass of land, and also many islands, having a very high percentage of endemic species. As an illustration of this, we may state that there are 117 known European species, only eighteen of which extend to other countries; and even amongst the European species, a considerable number are restricted to narrow areas. The half-dozen European species having a wide distribution beyond Europe are just those most associated with agriculture, occurring in farm-yards, dung-hills, &c., and have in all probability accompanied man in his migrations to new countries, whence they are recorded as occurring in similar habitats.

There is no differentiation of the hyphae into a laticiferous system; an entire absence of clear, bright colours, as also of those acrid and poisonous protective secretions which characterize the members of the higher Agaricineae. Certain species of *Coprinus*, as *C. comatus* and *C. atramentarius*, are edible, and amongst the safest and best of edible Fungi, and probably every species is edible, only owing to their ephemeral nature, and the absence of flesh, they do not commend themselves. The primary veil, when present, exhibits a primitive structure, not being sufficiently firm in texture to form a loose, sheathing volva at the base of the stem, and the portion carried upon the pileus usually consists of evanescent squamules, or a cobweb-like layer which soon vanishes. The thin flesh of the pileus, like that of the gills, deliquesces at maturity.

Notwithstanding the primitive simplicity of structure, it is highly probable that the five types of progressive development explained as present in the Agaricineae were also

originally present in *Coprinus*, although the two oldest types are obsolete, so far as is known, at the present day.

Throughout this paper, *Coprinus* has been spoken of as a genus; and from a systematic standpoint it is perhaps best to continue doing so, although its diagnosis, as already stated, is much broader than that of any other modern genus: for example, we have combined species with free and with adnate gills respectively; some species with a distinct universal veil, others without a trace of this structure, &c.; the only common bond is the deliquescent gills.

The section Melanosporae of modern Agarics is most closely allied to *Coprinus*, from which it is directly derived; in fact, numerous species belonging to this group differ from *Coprinus* only in having dry, persistent gills and wind-dispersed spores. But, as would be expected, we find in the Melanosporae many species in which the gills show a more or less decided tendency to deliquesce; as specific examples may be mentioned, *Hypholoma hydrophilus*, Bull; and *Agaricus campestris*, L., the common mushroom. The genus *Psathyrella*, in the Melanosporae, is in the sum-total of its characters nearest to *Coprinus*. In the Ochrosporae the genus *Bolbitius* approaches *Coprinus* in the ephemeral nature of its species, and in the partial deliquescence of the gills, but the spores are orange-brown in the mass.

Finally, in the Leucosporae, the genus *Hiatula* agrees with the simplest forms of *Coprinus* in the exceedingly thin flesh of the pileus, and in the gills splitting down the back; but the gills remain dry, and the spores are disseminated by wind.

In the descending series, *Coprinus* joins on to such genera as *Montagnites* with its three species, two of which are European, the third from Texas: *Phellorinia*, three species, S. Africa and Mongolia: and *Gyrophragmium*, one species from S. Africa. The two last-named genera have been located respectively in the Agaricineae and in the Gastromycetes by different authors, indicating that they are near the point of bifurcation of the two groups from the ancient subterranean basidiosporous Fungi.

It is interesting to note that, while the liquefaction of the elements of the hymenium—trama, basidia, paraphyses—was finally abandoned at a very early stage in the evolution of the Agaricineae, it persisted throughout the entire sequence of development in the parallel group of the Gastromycetes. In the puffballs — *Bovista* and *Lycoperdon* — the well-known water-logged condition of the immature Fungus is due to the melting of the hymenial structures, the spores being thus set free in the gleba, and after attaining maturity become dry, and are eventually dispersed by wind; whereas in the highest order, the Phalloideae, a similar deliquescence takes place, the semi-liquid product having a very decided smell and a sweet taste, much appreciated by insects, who greedily consume it along with the very minute spores imbedded in it; thus the feature which proved a failure in the Agaricineae has been an important factor in raising the Phalloideae to their present position as head of the fungal sub-kingdom.

Distribution.

The genus is cosmopolitan, species being most abundant in temperate regions; at the same time subtropical and tropical regions yield their own peculiar forms. Two common European species, *Coprinus fimetarius* and *C. micaceus*, are recorded from Siberia. Three species, *C. Barbeyi*, *C. imbricatus*, and *C. jasmundianus*, are peculiar to the Egyptian desert-region, growing on dung on caravan-tracks. Some species grow in absolute darkness, on timber in the shafts of coal-mines, &c., and very frequently under such conditions assume peculiar and grotesque forms, some of which have needlessly been raised to specific rank. The different sections of the genus, morphologically considered, are not characteristic of any particular region, as in those instances where a sufficient number of species are recorded from any extra-European country, the different types of structure are represented. An apparent exception to this statement is presented by the known distribution of the members constituting the most

highly organized section of the genus, characterized by the presence of a volva having a distinct free margin. There are twelve such species known, eleven of which are confined to Europe, the twelfth—*Coprinus jasmundianus*—being restricted to the Egyptian desert-region. Again, the species furnished with a ring or annulus on the stem are most abundant in Europe; but it must be remembered that both volva and ring are very evanescent structures, usually completely disappearing before the Fungus arrives at maturity, and at best are difficult to distinguish with certainty in the case of dried specimens; hence our knowledge of the subject is as yet too incomplete to justify the statement that the most highly organized species are most abundant in Europe; nevertheless, as shown by the following table, Europe has by far the largest percentage of endemic species.

The following arrangement shows the general and relative distribution of the species, the total number of which is 169.

Europe;	total of species,		117;	endemic species,		99.
Asia	„	„	12;	„	„	7.
Africa	„	„	16;	„	„	9.
Australasia	„	„	17;	„	„	5.
America	„	„	45;	„	„	31.

The particular country or district in each continent having the greatest number of species is given below:

France;	total of	species,	70;	endemic species,		24.
Ceylon	„	„	8;	„	„	6.
S. Africa	„	„	9;	„	„	2.
W. Australia	„	„	14;	„	„	3.
U. States	„	„	33;	„	„	20.

Great Britain follows France with a total of forty-three species, six of which are endemic. The following countries and islands have each one or more endemic species:—Abyssinia, Java, New Zealand, India, Bonin Is., Canary Is., Falkland Is., Venezuela, Brazil, Martinique, Cuba, Cayenne, St. Thomas (W. Indies), Tahiti, Tonga Island.

Coprinus plicatilis, Fries, a small, ephemeral Fungus, having a membranaceous pileus about 1·5–2 cm. across, when expanded, has the widest range of any known species; being common throughout Europe, also extending to South and West Africa, India, Ceylon, New Zealand, Tasmania, West Australia, United States, Japan, and Behring Straits.

Coprinus comatus, *C. atramentarius*, *C. niveus*, and *C. ephemerus* are also widely distributed.

Fungi belonging to the genus *Coprinus* are included in the figures of Japanese plants drawn by native artists, but the exact species cannot be determined with certainty.

Habitats.

Many species grow on dung or on richly manured ground, and are consequently most abundant in pastoral and agricultural districts, where they find a congenial home in farmyards, on dung-hills, &c. Other species grow on decaying tree-trunks, or at the base of old gate-posts, and similar localities. One species—*Coprinus radians*—possessing well-marked characters, has hitherto been met with only on old damp plastered walls, where the mycelium radiates on every side for a distance of 2–4 cm. from the point of attachment. Finally, a *Coprinus* has, both in this country and on the continent, been found growing on the dressing of wounds, an occurrence no longer possible, thanks to the researches of Sir Joseph Lister.

Classification.

It may perhaps be considered superfluous to give descriptions of species that have been previously described. The following are my reasons for so doing; and, furthermore, I consider these same reasons applicable in every instance where a genus has not been recently monographed.

Until quite recently many authors have attached primary importance to one special feature in the discrimination of

species, and in their diagnoses have emphasized this, to the comparative exclusion of other characters. Unfortunately there appears to have been no common agreement as to what constituted a feature of primary importance, hence the difficulty, or often the impossibility, of gaining a clear idea of the sum-total of characters considered necessary for the discrimination of a species at the present day, from the very brief descriptions of the old authors, and unfortunately also of some quite modern ones. A glance at almost any page of Saccardo's Sylloge Fungorum, which is supposed to give the original description of every described species of Fungus, will illustrate my meaning. It will there be seen that whilst the description of one species may not occupy more than two lines, that of the next species may occupy a dozen or more lines. Of these, the first would in all probability prove to lack points absolutely necessary for the certain identification of the intended species; whereas the second, on the other hand, might possibly prove to be a detailed description of an *individual*, as difficult to interpret correctly as the shorter one. Difficulties of the nature indicated are by no means absent from the descriptions of species, formulated by so many different individuals, and at different periods, in the genus *Coprinus*.

The following synopsis aims at keeping a certain number of features in view throughout the entire number of species, as follows:—(1) Form of pileus, especially when young; (2) presence or absence of volva and ring, and nature of universal veil when broken up by expansion of pileus; (3) mode of attachment of gills; (4) spores; (5) stem-characters. Unfortunately it is very rare to find all these features noticed in existing diagnoses, but in many instances I have been enabled to fill up omissions from examination of authentic or type specimens in the Herbarium of the Royal Gardens, Kew, and also in some cases from type-specimens kindly lent from other collections.

Key to the species of Coprinus.

SECTION I.

Volva distinct, with a free margin; ring present or absent. 1–12[1].

(The volva is in all cases more or less free from the base of the stem, and when the margin of the volva is persistent the ring is absent; in other words, the ring in all cases, when present, consists of the free margin of the volva, which breaks away, and is often carried away from the base with the elongating stem. No known species of *Coprinus* has a secondary veil, from which a true ring or annulus is formed.)

* Large; pileus always more than 2 cm. high and wide. 1–6.

† Stem white. 1–4.

†† Stem coloured. 5–6.

** Small; pileus always less than 2 cm. high and wide. 7–12.

† Volva entire. 7–10.

†† Volva torn into shreds. 11–12.

SECTION II.

Volva absent, ring present on the stem. 13–25.

(The statement that the volva is absent is morphologically inaccurate; it is present, but closely adnate to the stem which elongates much at the basal portion, the ring eventually occupying a more or less median position on the stem, the portion below the ring being in reality sheathed by the delicate volva—or peronate, as it is usually termed. This is sometimes very evident.)

* Large; pileus 8–15 cm. high. 13–17.

** Small; pileus never exceeding 3 cm. in height. 18–25.

[1] The figures correspond to the numbers preceding the names of species in the descriptive portion.

SECTION III.

Volva and ring absent. Veil practically absent, pileus either glabrous, or with minute innate squamules, especially near the apex. 26–42.

(Usually large; closely allied to the last section, differing only in the ring being obsolete. Differs from the glabrous group in Section VI in the pileus not splitting along the lines of the gills.)

* Gills attached to the stem. 26–34.

** Gills free. 35–42.

SECTION IV.

Volva and ring absent; veil very evident—at least in the young state—under the form of a felt-like layer, which breaks up during expansion into irregular patches; cottony; squamulose; fibrillose; or mealy (but not glistening and micaceous). 43–101.

(The presence of a universal veil, and the entire absence of volva and ring, characterizes this, the largest section of *Coprinus*. The presence of a universal veil implies the existence, at some stage, of a volva, but there is no trace of the latter even during a very young stage, hence for convenience it is described as absent, as in many other genera in the Agaricineae.)

* Veil rather thick and felty, breaking up into irregular, more or less persistent, patches during the expansion of the pileus. 43–49.

† Gills attached to the stem. 43–44.

†† Gills free. 45–49.

** Veil breaking up into superficial scales, cottony, or fibrillose. 50–92.

† Gills attached to the stem. 50–68.

§ Pileus whitish or grey. 50–60.

§§ Pileus tawny or brownish. 61–68.

†† Gills free. 69–92.

§ Stem glabrous. 69–78.

§§ Stem floccose or pulverulent at first. 79–92.

*** Veil formed of white meal or hyaline vesicles (not glistening and micaceous). 93–101.

Section V.

Volva and ring absent. Pileus covered with glistening, micaceous particles when young. 102–110.

(It is uncertain as to whether traces of a veil are in reality present on the pileus of the species constituting the present section. The glistening particles of oxalate of lime are washed away in rainy weather, leaving the pileus naked.)

* Gills attached to the stem. 102–106.

** Gills free. 107–110.

Section VI.

Volva, ring, and veil entirely absent; flesh exceedingly thin, and the pileus soon becomes radially fissured or split along the lines of the gills; furfuraceous or scurfy, or glabrous. 111–165.

(The leading character of the present section consists in the radial splitting of the pileus along the lines of the back of the gills during the expansion of the pileus. In all the previous sections, the radial splitting of the pileus takes place along the lines of striation, which are situated between the gills. The furfuraceous or scurfy appearance is not due to the presence of a veil, but is caused by the cells of the thin flesh which are torn apart, and project outwards when the splitting takes place. In those species where the flesh of the pileus is reduced to a mere film, the surface remains glabrous after splitting.)

* Pileus more or less furfuraceous or scurfy. 111–131.

† Gills attached to the stem. 111–120.

§ Pileus white. 111–113.

§§ Pileus coloured. 114–120.

†† Gills free. 121–131.

§ Stem fibrillose or downy. 121–122.

§§ Stem glabrous. 123–131.

** Pileus glabrous. 132–165.
† Gills attached to the stem. 132–145.
§ Stem downy or pulverulent. 132–134.
§§ Stem glabrous. 135–145.
†† Gills free. 146–157.
§ Stem downy or pulverulent, 146–148.
§§ Stem glabrous. 149–157.

COPRINUS, Fries.

Pileus symmetrical, flesh thin, usually radially striate or grooved, and bearing the remains of the universal veil; stem central, most frequently hollow, in some species volvate and annulate; gills adnate, adnexed, or free, thin, crowded, deliquescing at maturity, trama well developed; basidia clavate or piriform, tetrasporous; spores large, elliptical or irregularly angular, black, often with a tinge of purple or brown when seen in the mass; cystidia numerous.

Coprinus, Persoon, Syst. Meth. Fung., p. 395 (1801); used as a sectional name in the genus *Agaricus*.

Fries, Epicr. Syst. Mycol., p. 241 (1836–1838).

SECTION I.

1. **Coprinus sterquilinus**, Fries, Epicr. p. 242.

Pileus conic-ovate, then campanulate, *coarsely sulcate*, silvery-grey, *disc tawny and covered with squarrose squamules*, 5–7 cm. high; gills free, purplish-black; spores 18–20 × 11–12 μ; stem 9–15 cm. high, fibrillose, white, becoming *dark coloured when bruised*, volva adnate, margin free, sometimes carried up by the elongating stem as a ring.

On dung and manured ground. Britain, France, Germany, Spain, Portugal, Sweden, Belgium.

Distinguished among volvate species by the squamulose disc, and the white stem becoming blackish when bruised.

2. **Coprinus solstitialis**, Sacc., Syll. Suppl. 3, no. 498.

Pileus cylindric-ovate, 2·5–3 cm. high and 1·5 cm. broad, even, whitish, *covered with spreading, overlapping, somewhat concentrically*

arranged scales, with an unbroken patch at the disc, soon expanding, becoming blackish, coarsely grooved, and up to 5 cm. across; gills free; stem 3–6 cm. high, white, base volvate and with a fugacious ring formed by the margin of the volva breaking away.

On sandy ground. Finland.

Allied to *C. sterquilinus*, differing in being smaller, and in the stem not becoming black when bruised.

3. **Coprinus oblectus,** Fries, Epicr. p. 243.

Pileus cylindric-ovate, then campanulate, coarsely striate, at first whitish and silky, *glabrous*, pale tawny, and *sprinkled with rose-coloured powder*, 3–5 cm. broad; gills free, pinkish, then black with a purple tinge; spores broadly elliptical, 18 × 11–12 μ; stem 8–12 cm. long, persistently white, silky, volva with a broad, recurved margin.

On dung, road-scrapings, &c. England, France.

4. **Coprinus jasmundianus,** Kalchbr., in Asch. Beitr. Flor. Aeg., 1879, p. 73.

Pileus conic-ovate, grooved, sordid, *flocculose*, 4–5 cm. broad; gills linear, black; stem 10–12 cm. long, whitish, *striate*, hollow, base bulbous, volvate.

On the ground on caravan-tracks. Egypt.

5. **Coprinus stenocoleus,** Lindblad, in Fries, Mon. p. 306.

Pileus cylindrical, then broadly campanulate and umbonate, *even*, blackish, with white squamules, 5–8 cm. across; gills free; stem 10–15 cm. long, slender, pale tawny, more especially upwards, base thickened and closely sheathed by a long volva having the margin free.

On manured ground. Sweden.

6. **Coprinus umbrinus,** Mass., Grev. Vol. xxi, p. 41. (Figs. 13–14.)

At first entirely enclosed in a white volva; pileus conico-hemispherical, soon almost plane, *coarsely sulcate* up to the disc, umber, ornamented with patches of the volva, 4–5 cm. across; gills free; spores elliptic-oblong, 17–18 + 9 μ; stem 10–15 cm. long, dark umber from the first, base bulbous, with a persistent white volva having a free, reflexed margin.

On manured ground. England.

Distinguished from *C. stenocoleus* by the sulcate pileus and umber stem.

7. **Coprinus cyclodes**, Fries, Epicr. p. 250.

Pileus ovate, then campanulate, striate, *glabrous*, *bay*, *disc darker*, 1·5–2 cm. high; stem 4–5 cm. long, 3–4 mm. thick, rather flexuous, white, base sheathed in a volva having a free, recurved margin; gills white, then black.

Gregarious; on horse-dung. Italy, France, Hungary.

8. **Coprinus equinus**, Chelch., Mem. Phys. de Vars. p. 6, t. xi, f. 11.

Pileus ovate, then campanulate, greyish-white, disc darkest, *covered with darker scurf and flecks*, 3–15 mm. broad; *gills free*; spores 5–6 and 4–5 μ; stem 18–35 mm. long, glabrous, base rather swollen, and enclosed in a volva with a free margin, margin sometimes breaking away as a ring.

On horse-dung. Poland.

9. **Coprinus Trappenii**, Oudem., Arch. Néerl. ii, p. 29.

Pileus ovate, then campanulate, apex at first bearing fragments of the volva, then glabrous, 1–1·5 cm. across; gills *purplish*, then black; stem 1–1·5 cm. high, glabrous, base sheathed with a volva, white.

Growing on twigs. Netherlands, Holland.

Distinguished by growing on fallen twigs.

10. **Coprinus volvaceo-minimus**, Crossl., Grev. Vol. xxi, p. 69.

Pileus ovate, then campanulate, striate, grey, disc darker, *sprinkled with white squamules*, 4–5 mm. across; *gills slightly adnexed*; spores 6–7 μ; stem 2–2·5 cm. long, glabrous, hyaline, the bulbous base having a volva with a broad, persistent, free, spreading margin.

On a manure-heap. England.

Distinguished from *C. Hendersonii* by the distinct volva and smaller subglobose spores. May prove to be identical with *C. bulbillosus*, Pat.

11. **Coprinus dilectus**, Fries, Epicr. p. 250.

Pileus cylindrical, then campanulate, finely striate, rosy-white, then pale tawny, floccose or mealy, 1–2 cm. broad; gills free, reddish brown, then black; stem 5–7 cm. long, whitish and *sprinkled with red powder*; volva reduced to whitish spreading squamules at the base of the stem.

On scorched ground, in plant-pots, &c. Sweden, France.

Agreeing with *C. oblectus* in being sprinkled with red powder, but differing in the finely striate pileus and the much reduced volva.

12. **Coprinus panormitanus**, Inzenga, Fung. Sicil. ii, p. 58, tab. x, fig. 1.

Pileus ovate, white, then grey with an olive tinge, and sprinkled with whitish flecks, even, 1·5 cm. high; gills close to the stem; spores ovate, black; stem not longer than pileus, base swollen, rooting, furnished with a torn, *ochraceous* volva.

On damp ground. Sicily.

Judging from the description and figure, the species was founded on immature, unexpanded specimens.

Section II.

13. **Coprinus comatus**, Fries, Epicr. p. 242.

Pileus cylindrical, then campanulate, whitish or tinged ochraceous, at first even, then *becoming broken up into scattered, more or less reflexed, large torn scales*, soon becoming campanulate and pinkish grey at the margin, 9–15 cm. high; gills very slightly adnexed, pink, then blackish; spores 12–14 × 8–10 μ; stem 12–20 cm. high, stout, white, volva usually evanescent, its free margin forming a ring which is often carried up for some distance by the elongating stem.

Gregarious on rich soil in gardens, pastures, &c. Britain, Germany, Holland, Spain, Portugal, Denmark, Sweden, France, Italy, Belgium, Russia, Finland, Hungary, Switzerland, Austria, Cape of Good Hope, Himalayas, Japan, Western Australia, Tasmania, New Zealand, United States.

Among the best and safest of edible Fungi.

Var. **ovatus**, Quél., Enchirid. p. 121 (=*Coprinus ovatus*, Fries, Epicr. p. 242).

Pileus ovate at first, covered with overlapping, concentrically arranged scales; gills free.

On rich soil, among grass, &c.

Rather smaller than the type form.

Met with in most countries where the typical form occurs, but not recorded from any country where the type-form is unknown.

Var. **clavatus**, Quél., Enchirid. p. 121 (=*Coprinus clavatus*, Fries, Epicr. p. 242).

Pileus ovate, soon expanding, surface broken up into shaggy,

recurved scales, almost from the first; gills free, white, then blackish, without any intermediate red colour; volva without a free margin, hence the ring is absent.

Rather smaller than the type-form.

14. **Coprinus atramentarius**, Fries, Epicr. p. 243.

Pileus rather fleshy, ovate, then campanulate, irregularly fluted, margin uneven, greyish white, with minute brownish squamules near the apex, 8–12 cm. high; gills free, broad, *white, then purplish-brown*; spores 12 × 6 μ; stem 10–16 cm. long, white, *hollow*; ring basal, very evanescent.

About old stumps and on rich soil; not on dung. Britain, France, Germany, Sweden, Italy, Portugal, Holland, Spain, Denmark, Russia, Belgium, Finland, Hungary, Switzerland, Austria, United States, Cape of Good Hope, Kerguelen Island.

Caespitose; large, edible. Distinguished from *C. praegnans* and *C. soboliferus* in having the stem distinctly hollow.

15. **Coprinus soboliferus**, Fries, Epicr. p. 243.

Pileus thin, ovate, then expanded, lower half plicate, disc truncate, often depressed, whitish grey, apex brownish and bearing darker brown squamules, 9–12 cm. high; gills free, broad, *white becoming black*; spores elliptical, 15 × 7 μ; stem 12–20 cm. long, white, *stuffed*; ring fugacious.

On the ground near trunks and buried wood. Sweden, Britain, Germany, Holland, France, Hungary.

Clustered. Distinguished from allies by the stuffed stem and truncate disc of pileus. Very close to *C. atramentarius*, if indeed distinct as a species.

16. **Coprinus pyrenaeus**, Quél., Assoc. Franc. 1887, p. 2, pl. xxi, fig. 6.

Pileus narrowly elliptical, up to 10 cm. long, striate, pearl-grey showing through *a dense veil of free white fibrils*; gills free, pinkish, then brown; spores irregular, ovoid to almost globose, 12–18 μ long; stem 10–15 cm. long, hollow, white, fibrillose, ring narrow, fugacious, basal.

On the ground in troops in alpine regions. France.

Distinguished from *C. atramentarius* by the dense white veil.

17. **Coprinus praegnans**, Fries, Epicr. p. 243.

Pileus campanulate, rather fleshy, not striate but *everywhere covered with crowded, minute squamules*, cinereous; gills free, broad, *umber from the first*; stem fusiform, rooting, *solid*, fibrillosely squamulose, ring free.

On the ground. Sweden.

Large, 18–20 cm. high. Allied to *C. atramentarius*, differing in the gills not being white, then purplish, but umber from the first, solid stem, &c.

18. **Coprinus variegatus**, Peck, 25th Rep. New York State Mus. p. 79.

Pileus oblong-ovate, then campanulate, obtuse, *hygrophanous, brown, then whitish, variegated with ochraceous tomentose scales*, margin slightly striate, 2·5–4 cm. broad; gills free and ascending, white then rosy, at length black; spores elliptical, 9 μ long; stem equal, fragile, hollow, at first peronately-annulate, ring soon disappearing, then floccosely pruinose, 8–13 cm. high.

Among dead leaves. United States.

Distinguished from its ally *C. atramentarius* by being enveloped in a floccose veil when young, which later becomes broken up into scales.

19. **Coprinus armillaris**, Fries, Nov. Symb. Myc. p. 28.

Pileus *pellucid*, conical, becoming plane, *sulcate*, whitish, disc greyish, 2·5 cm. across; gills at first white; stem 5–7·5 cm. long, narrowed upwards from a *ventricose base, rufescent and slightly squamulose below*, with a small, entire, median ring.

Island of St. Thomas, West Indies.

20. **Coprinus Hendersonii**, Fries, Epicr. p. 250.

Pileus subcylindrical, then almost plane, margin slightly fluted, *minutely pruinose*, apex tawny, remainder greyish white, up to 1 cm. across; gills free; spores elliptic-oblong, 10–12 × 6 μ; stem 3–4 cm. long, with *a permanent ring below the middle.*

On the ground. Britain, France, Belgium.

There is at times distinct evidence of the stem being peronate up to the ring. Allied to *C. bulbillosus*, differing in the elliptical spores, and absence of a bulb at base of stem.

21. **Coprinus bulbillosus**, Pat., Tab. Anal. Fung., p. 60, fig. 658.

Pileus convex with the margin striate and incurved, then expanding, grey with the disc tinged yellow, *covered with white meal*, 8–10 mm across; gills grey; spores oval, 8–9 × 6–7 μ; stem 2–3 cm. long, slender, white, *base bulbous*, ring loose, central on the stem.

On horse-dung. France.

Differs from *C. Hendersonii* in the bulbous stem. *C. ephemeroides* differs in the squamulose pileus and strigose bulb. See note under *C. volvaceo-minimus*.

22. **Coprinus ephemeroides**, Fries, Epicr. p. 250.

Pileus cylindric-ovate, then campanulate, *plicato-sulcate*, whitish or livid, disc tinged yellow, *sprinkled with superficial flecks*, up to 1 cm. high and broad; gills free, distant from the stem; spores elliptical, 11–12 × 6–7 μ; stem 2–4 cm. long, whitish, with a free ring usually placed some distance up the stem, *base with a pilose bulb*.

On dung. France, Germany, Holland, Sweden, Finland.

Variable in size, but always small and delicate.

Var. **muscorum** (= *Coprinus muscorum*, Karsten, Hattsvamp. 1, p. 531.

Spores ovoid, 7–9 × 6–8, fuscous.

Among dead moss. Finland.

23. **Coprinus torquatus**, Mont., Cent. vii, no. 29.

Pileus very delicate, soon plane, *centre slightly depressed*, *pellucid*, striate up to the even, livid disc, *pale grey*; gills free and distant from the stem, very narrow; stem long, slender, base bulbous, *ring funnel-shaped*, *entire*, whitish.

Solitary. On the ground in damp, shady spots. Brazil.

24. **Coprinus scauroides**, Godey, in Gillets' Champ. France, Hymen. p. 609.

Pileus ovate, then campanulate, striate, *floccosely squamulose*, *white*, *then purplish*, soon black with the disc yellowish; gills free, purplish, then black; spores ovate, black; stem silvery-white, with a *marginate bulb* and a ring.

On manured ground. France.

25. **Coprinus Bresadolae**, Schulz., Hedw. 1885, p. 136.

Pileus subcylindrical, *greyish white*, *apex tinged brown*, 17 mm. high

by 8 mm. across; gills black, edge white; spores cylindrical, ends rounded, black, 12–17 × 6 μ; stem up to 12 *cm. long*, base 4 mm. thick, tapering upwards, furnished with a loose, deciduous ring, white, glabrous.

Gregarious; on worked wood. Hungary.

Always expands at night, becoming diffluent as it does so. At first covered with a very thin, universal veil, which does not break up into squamules, but splits from apex to base, and becomes obliterated.

Section III.

26. Coprinus fuscescens, Fries, Epicr. p. 244.

Pileus thin, ovate then expanded, striate, margin not lobed, disc rufous, sometimes breaking up into scales, remainder greyish brown, powdered at first with opaque meal, 4–6 cm. high and broad; gills adnexed, *narrowed towards the front*; spores 8–10 × 5–6 μ; stem 8–12 cm. high, fragile, white, hollow, often curved, *slightly fibrillose.*

On trunks and stumps. Britain, France, Germany, Finland, Holland, Sweden, Belgium, United States, Argentine Republic, Ceylon, Victoria.

Allied to *C. atramentarius*, but smaller, pileus with more of a rufous tinge and not so irregular, and gills gradually narrowing from stem to margin.

27. Coprinus insignis, Peck, 26th Rep. p. 60.

Pileus thin, campanulate, *sulcately-striate up to the disc*, greyish fawn-colour, disc glabrous, sometimes cracking into areolae, 5–8 cm. across; gills ascending; *spores asperate*, 10 × 8 μ; stem pure white, *striate*, hollow, 10–13 cm. long.

Near roots of trees in woods. United States.

Size and general aspect of *C. atramentarius*, but distinguished from this and all other allies by the rough spores.

28. Coprinus imbricatus, Rabenh., Hedw. 1871, p. 25.

Conic-ovate, then campanulate, white, *covered with large, imbricated, concentrically arranged, tawny-white scales*, 3·5–5 cm. across; gills *adnate and subdecurrent*; spores elliptical, 18–21 × 13–14 μ; stem white with a tawny tinge, hollow, *striate*, 5 cm. long.

Among sand. Mesopotamia.

29. **Coprinus Barbeyi**, Kalchb., Rev. Mycol. Vol. iii, n. 90, p. 24, tab. 15, f. 1 (1881).

Pileus sub-hemispherical, then expanded, *covered with large, persistent, imbricated, pale tawny scales*, 2·5–5 cm. broad; gills uncinately adnate; spores 13–20 μ long; stem about 5 cm. high, hollow, white with a tawny tinge, *ending in a dilated disc*, below which the mycelium collects the sand and forms an inverted cone.

On camel's dung in sandy desert. Egypt.

This species appears to be identical with *C. imbricatus*, Rab.

30. **Coprinus tergiversans**, Fries, Epicr. p. 247.

Pileus conical, then expanded, silky, soon grooved, cracked up into minute squamules, rusty brown, disc darker, even, 6–12 cm. broad and high; gills *broadly adnate*; spores 10 × 4 μ; stem white, equal, glabrous, *apex sulcate*, 10–14 cm. long.

Caespitose; in rich meadows. Sweden, France, Germany, Holland, Belgium.

Allied to *C. micaceus*, but the pileus is rather more fleshy, darker in colour, and not micaceous, but covered with minute, wart-like squamules.

31. **Coprinus Lerchenfeldii**, Schulz., Verhandl. Hermann. 1884, t. 1, f. 3.

Pileus hemispherical, apex elevated, brownish grey, margin undulated, *fimbriate*, silvery grey, then violet, 5–7·5 cm. broad; gills violet, shining; stem 12–15 cm. long, *fibrillose, or squamulose.*

In gardens. Austria.

32. **Coprinus pauci-lamellatus**, Pat., Journ. Botanique, 1889, p. 165.

Pileus thin, campanulato-convex, apex obtuse and squamose, yellowish, remainder white; margin entire, even; gills few in number (20–25), *very distant*; spores lemon-shaped, 15–20 × 10–12 μ; stem 10–12 cm. long, 5 mm. thick, cylindrical, white, *striate the entire length.*

On dung. Venezuela.

33. **Coprinus musicola**, Berk., Hook. Lond. Journ. Bot. Vol. i, p. 453.

Pileus companulate, pale *purple-brown*, surface broken up into squamules, about 1·5 cm. high and broad; gills adnexed; spores

broadly elliptical to subglobose, minutely apiculate, 8–10 μ; stem 2·5–3 cm. high, slender, *pale purple-brown*, pulverulent.

On the stem of a *Musa*. Tahiti.

34. **Coprinus fibrillosus**, B. & Br., Linn. Soc. Bot. Journ. Vol. xi, p. 560.

Pileus ovate, *even*, grey with persistent fibrillose, darker scales, about 1 cm. across; gills adnexed, fuscous; spores elliptical, 5–6 μ long; stem 2–3 cm. high, curved, slightly *floccose*, white.

On the ground. Ceylon.

35. **Coprinus cylindricus**, Fries, Epicr. p. 244.

Pileus *cylindrical*, then expanded, rimosely striate, with a few scattered, adpressed squamules, 8–12 cm. across when expanded, *whitish brown*; gills free, rather narrow; stem 15–21 cm. long, *equal, fibrillose*.

On the ground near trunks. Germany, Sweden.

36. **Coprinus Mayrii**, Allesch., Sued-bayr. Pilze, p. 102.

Pileus campanulate, then expanded, white, rather coarsely striate, *with small yellow-brown squamules near the margin*, disc sparingly scaly, 6–8 cm. high; gills free, broad, lanceolate; spores 6–7 × 3–4 μ; stem 6–8 cm. high, white, *striate*, base globose, marginate, hollow to the swollen base.

Solitary. On trunks and rotten wood. Bavaria.

Allied to *C. atramentarius*.

37. **Coprinus saatiensis**, Henn., Engler's Bot. Jahrb., Vol. xiv, p. 352.

Pileus fleshy, at first cylindric-ovate, covered with *imbricated, concentric, white lacerated scales*; then expanding, sooty black, variegated with scattered, broad white scales, 5 cm. diameter; gills free; spores 19–23 × 10–12 μ; stem 8 cm. long, 1 cm. thick, cylindrical, base bulbous, whitish, then *tinged fuscous*, hollow.

On the ground. Abyssinia.

38. **Coprinus punctatus**, Kalchbr. & Cooke, Grev. Vol. ix, p. 18. (Figs. 35–36.)

Pileus cylindrical, then companulate, 2·5–3·5 cm. broad, brownish, margin striate, *apex depressed and squamulose*, remainder punctate with minute black squamules and vaguely cracked; gills free, narrowed

behind; spores 15 × 10 μ; stem 15–20 cm. long, solid, fibrillose, pallid, *ventricose at the middle, base bulbous.*

On the ground. Cape of Good Hope.

39. **Coprinus flocculosus**, Fries, Epicr. p. 245.

Pileus *ovate*, then expanded, *dirty white*, *striate*, with innate squamules, 4–7 cm. across; gills free, narrow; spores 10 × 7–8 μ; stem 6–10 cm. high, white, *silky*, *shining*, hollow.

On the ground in fields, &c. Britain, France, Sweden.

Solitary or tufted. Allied to *C. aratus* and *C. lagopus*. Differs from the former in the white pileus, and from the latter in the smooth stem and disc of pileus not being brown.

40. **Coprinus stenophyllus**, Mont., Syll. Plant. Crypt. no. 410, p. 132.

Pileus ovoid-campanulate, soon expanded, slightly striate, *broken up into scattered ochraceous scales*, rufous, becoming fuliginous, 5–7 cm. diameter; gills free, but adpressed to the stem, *very narrow*; stem cartilaginous, elongated, hollow, smooth, white.

On the ground among rotten wood. United States.

Allied to *C. deliquescens* and *C. micaceus*, differing from both in the very narrow gills and scaly pileus.

41. **Coprinus microsporus**, B. & Br., Linn. Soc. Journ. (Bot.) Vol. xi, p. 560. (Fig. 59.)

Pileus campanulate, obtuse, *dirty white*, *with scattered innate*, *pale umber scales*, about 1·5 cm. across; gills free, white with a red tinge, slowly becoming black; *spores* 4–5 × 2 μ; stem 3–4 cm. high, white, smooth, curved, hollow.

On soil. Ceylon.

42. **Coprinus macrosporous**, Peck, 31st Rep. N. York State Mus. p. 35.

Pileus ovate, then expanded, rimoso-striate, *obscurely floccose-squamulose*, *white*, *the small*, *even*, *brownish disc squamose*, 2·5–5 cm. across; gills free, white, then black; spores elliptical, 20–25 × 13–15 μ; stem 5–7·5 cm. high, white, glabrous (with traces of a ring near the thickened base).

Caespitose; ground in open fields. United States.

The prominent characters of this species are the rimose pileus, squamose disc, free gills, and large spores.

SECTION IV.

43. **Coprinus aphthosus**, Fries, Epicr. p. 245.

Pileus ovate, then campanulate, *even*, *livid*, at first involved in a continuous white veil, which becomes broken up into superficial, floccose patches, about 2·5 cm. high and broad; *gills adnate*, spores 15–16 × 10 μ; stem about 5 cm. long, white, *fibrillose*, *hollow*, *soft*, often twisted.

In hollow trunks. Britain, Sweden, France.

Growing in small clusters. Differs from *C. varicus* in the hollow, soft stem.

44. **Coprinus phaeosporus**, Karst., Symb. Myc. Fenn. viii, p. 9, and ix, p. 48.

Pileus conico-cylindrical, then flattened, *everywhere delicately striate*, at first enclosed in a rufescent, floccose veil which breaks up into patches, soon naked and then white, about 2 cm. high; gills adnexed; spores 9–15 × 4–9 μ; stem 3–12 cm. long, *glabrous*, white, hollow.

On rich ground at roots of decaying grass. Finland.

Densely caespitose. Distinguished from *C. albus*, Quél., by the pileus not being flocculosely mealy and the glabrous stem.

45. **Coprinus picaceus**, Fries, Epicr. p. 244.

Pileus ovate, then campanulate, *glutinous*, *striate up to the disc*, blackish, at first involved in a white felty layer which becomes broken up into patches as the pileus expands, 4–7 cm. across; gills free; spores 14 × 8 μ; stem 10–15 cm. long, white, smooth, hollow, *base swollen*.

On the ground. Britain, Denmark, France, Italy, Germany, Belgium, Sweden, Spain, Portugal, Queensland, Ecuador, United States.

Distinguished by the black pileus having felt-like white patches scattered irregularly over its surface.

45*. **Coprinus ebulbosus**, Peck, Bull. Torrey Bot. Club, Vol. xxii, p. 491.

Pileus thin, campanulate, variegated by the cuticle breaking into broad, superficial, persistent, whitish scales, the surface beneath the

cuticle somewhat striate, *greyish brown*, the margin at length revolute, lacerated, 5–7·5 cm. broad; gills narrow, thin, crowded, free, slate colour, becoming black; spores about 10 × 5·5 μ, elliptical, stem 8–15 cm. long, 4–6 mm. thick, *equal*, hollow, white.

Caespitose at the base of cotton-wood stumps. United States.

This plant resembles *C. picaceus* very closely. New York specimens were formerly referred to it as variety *ebulbosus*, but now having received it from various widely separated localities and finding that it maintains its distinctive character with constancy, it seems best to consider it a good species. Its peculiar characters are the absence of a bulbous base to the stem and its smaller spores. It also sometimes grows in large tufts. 'About fifty grew in a solid clump, all united at the base' (Peck).

If the present plant is so closely allied to *Coprinus picaceus*, then the structure called the cuticle will in reality be the volva.

46. **Coprinus tomentosus**, Fries, Epicr. p. 246.

Pileus *cylindrical, then narrowly conical*, at length expanded, striate, entirely covered at first with a greyish felty veil which becomes torn into scales during expansion, pallid or yellowish below the veil, 2·5–3 cm. high; gills free; stem about 5 cm. long, *velvety*, greyish, hollow.

On dung and in rich pastures. Britain, Sweden, Switzerland, Holland, Spain, Portugal, Italy, Russia, Belgium, Finland, France, Germany, Ceylon, Queensland, Kerguelen's Land, Victoria, United States.

47. **Coprinus velatus**, Quél., Bull. Bot. Soc. Fr., Vol. xxiii, p. 329, pl. 2, fig. 6.

Enclosed at first in a thin, white volva; pileus cylindrical, then spreading, *coarsely furrowed, yellow or pale ochraceous*, 2–3 cm. across; gills free, but close to the stem; spores elliptical, 10 × 5 μ; stem 4–6 cm. long, rather stout, white, villose, *coarsely striate*.

In troops on the ground in woods. France.

48. **Coprinus Forquignoni**, Massee.

[*Coprinus Quéletii*, Forq., Bull. Soc. Bot. France, 1887, p. xxxi, pl. 1, fig. 1 (non Schulzer)].

Pileus ellipsoid, then conico-campanulate, at first enclosed in a thickish *ochraceous veil*, which becomes broken up during expansion

into irregular, persistent patches, whitish, then tinged grey, 5–6 mm. high; gills free, then remote; spores pip-shaped, 9 × 6 μ; stem up to 5 mm. long, pure white, slightly *floccosely-fibrillose, ending in a tawny bulb.*

Subgregarious on dead leaves. France.

Distinguished from *C. Friesii* and *C. tigrinellus* by the tawny, furfuraceous bulb at the base of the stem.

49. **Coprinus varicus**, Fries, Epicr. p. 244.

Pileus ovate, then campanulate, *white or livid towards the split margin*, covered with broad, irregular, persistent but superficial white patches of the universal veil, 4–5 cm. across; gills free; spores . . . ? stem 6–9 cm. long, often incurved, white, *glabrous, solid, rigid, tough.*

On diseased portions of living beech-trees. Sweden.

Allied to *C. picaceus*, but distinguished by the white pileus and solid, rigid, tough stem.

50. **Coprinus niveus**, Fries, Epicr. p. 246.

Pileus elliptical, then campanulate, *covered with snow-white, floccose down*, 1·5–2·5 cm. across; gills slightly adnexed; spores 16 × 11–13 μ; stem *villose, white*, hollow, 5–8 cm. high.

On dung, especially of horses. Britain, Sweden, France, Germany, Holland, Spain, Portugal, Denmark, Italy, Russia, Belgium, Finland, Hungary, Switzerland, United States, Victoria.

Distinguished by the snow-white colour of every part, the persistently floccose pileus and stem, and adnexed gills.

Var. **astroideus**, Fries, Epicr. p. 247.

Pileus squamose, then inversed, *naked, and grey*, about 12 mm. broad; stem up to 8 cm. long, slender, *glabrous*, base stellately strigose.

An imperfectly known form.

51. **Coprinus Colensoi**, Berk., Fl. N. Zeal. ii, p. 175.

Pileus cylindrical, then slightly campanulate, *densely covered with persistent white villose down*, grey below the down, and delicately striate, 3–4 mm. high; gills adnexed; spores 7–8 × 5 μ; stem slender, about 2 cm. high, *tomentose*, white.

On dung. New Zealand.

Resembling *C. niveus* in miniature.

52. **Coprinus albus**, Quélet, Assoc. France, 1880, p. 4.

Entirely snow-white; pileus ovoid, then expanded, floccosely mealy; at length grooved, pearly grey, and with tawny flecks at the summit, up to 2 cm. across; gills adnate, seceding; spores obliquely elliptical, 12–13 μ long; stem *coarsely striate upwards*, base swollen and downy.

Fasciculate; on decaying vegetable matter, &c. France.

Superficially resembling *C. Friesii*; allied to *C. stercorarius*.

53. **Coprinus pilosus**, Beck, Pilzfl. von Niederöst. iii, p. 44.

Pileus at first cylindrical, apex rounded, white, densely covered with septate, acute, hairs, when expanded centre almost glabrous and *yellowish*, margin slightly striate, 8 mm. broad; gills . . . ? spores elliptical, 9–12 × 6–7 μ; stem up to 5·5 cm. high, very slender, watery, minutely pubescent, *floccose at base*.

On sheep-dung. Austria.

54. **Coprinus exstinctorius**, Fries, Epicr. p. 245.

Pileus cylindric-clavate, then campanulate, margin striate, whitish, apex tinged brown, *clothed at first with evanescent, floccose scales*, 3–5 cm. across; gills reaching the stem; spores 10–11 × 6–7 μ; stem 8–12 cm. long, *smooth*, white, hollow, *swollen at the base and rooting*.

On the ground about the roots of trees. Britain, France, Holland, Germany, Sweden, Spain, Portugal, Italy, Russia, Belgium, Ceylon.

Allied to *C. fimetarius*, which differs in the pileus becoming bald from the margin to the disc, whereas the present species becomes bald first at the disc, the baldness progressively extending to the margin.

55. **Coprinus fimbriatus**, B. & Br., Linn. Soc. Journ. (Bot.) Vol. xi, p. 561.

Pileus campanulate, tomentose, whitish, *margin fringed with white hairs*, striate, 1–2 cm. across; *gills adnate*; spores 8 × 5 μ; stem 5 cm. long, *glabrous*, equal, white, hollow.

On dung. Ceylon.

Allied to *C. stercorarius*.

56. **Coprinus roris**, Quél., Bull. Soc. Bot. Fr., Vol. xxiv, p. 322, pl. 5, f. 5.

Pileus soon convex and *with the centre depressed, sulcate*, glaucous or pearly grey, covered at first with a thin, evanescent, tawny white veil,

transparent, 1–1·5 cm. broad; gills adnate; spores ovate, 11–12 μ long; stem up to 4 cm. long, slender, *greyish, villosely floccose.*

Among short grass. France.

Very fugacious, differs from *C. plicatilis* in the adnate gills, and from *C. diaphanus* in the villosely floccose stem.

57. **Coprinus Brassicae**, Peck, 43rd Rep. N. York State Mus. p. 18, pl. 2, fig. 9–14.

Pileus ovate or conical, then broadly convex, squamulose, *finely striate to the disc*, white, becoming greyish brown, 4–5 lines broad; gills narrow, reaching the stem, *brownish*; spores elliptical, brown, 7·5 × 5 μ; stem white, slender, hollow.

On decaying cabbage-stems. United States.

This species is easily known by its squamulose pileus, and its brown gills and spores. Allied to *Coprinus phaeosporus*, *C. Friesii*, and *C. tigrinellus*.

58. **Coprinus similis**, B. & Br., Ann. Nat. Hist. Ser. 3, Vol. xv, p. 317.

Pileus ovate, then campanulate, *striate* pallid, *disc darker*, *studded with brown-tipped pointed warts* which eventually disappear, about 2·5 cm. across; gills adnate; spores . . . ?; stem white, hollow.

On trunks of dead trees. Britain.

Resembling *C. aphthosus*, but differing in the striate pileus.

59. **Coprinus murinus**, Kalchbr., Grev. Vol. viii, p. 152, pl. 142, Fig. 10, in Vol. ix.

Pileus conico-campanulate, *prominently papillate*, sprinkled with white floccose squamules, scarcely striate, grey, up to 1 cm. high; gills adnexed; stem 1–3 cm. long, white.

On the ground. Victoria.

Allied to *C. coöpertus*, differing in being papillate, opaque, pulverulent, and not micaceous.

60. **Coprinus Brunandii**, Quél., Champ. Jura et Vosg. Suppl. xvii, p. 4, t. 15, f. 11.

Pileus campanulate, 5–6 mm. high, very delicate, striate, greyish lilac, covered at first with very delicate, *crystalline, interwoven, caducous*

filaments, 3–4 mm. long; gills adnate, becoming free; stem slender, white, *floccose*, hollow, *bulbous*; spores 10 μ long.

Gregarious on rotten leaves in damp places. France.

Allied to *C. lagopus*.

61. **Coprinus domesticus**, Fries, Epicr. p. 251.

Pileus ovate, then campanulate, obtuse, *sulcate*, *disc even*, *bay*, *remainder whitish or pale tawny*, furfuraceo-floccose, 4–7 cm. across; gills adnexed; spores elliptic-oblong, 11–12 × 7 μ; stem 6–9 cm. long, adpressedly silky, white.

On the ground among rubbish, &c. Britain, Germany, Sweden, France, Russia, Belgium, Switzerland, United States.

Very brittle, often caespitose. Larger than its allies, *C. stercoreus*, *C. ephemerus*, &c.

61*. **Coprinus laniger**, Peck, Bull. Torr. Bot. Club. Vol. xxii, p. 491.

Pileus thin, conical or campanulate, *covered when young with numerous tawny tomentose or floccose scales*, which partly or wholly disappear with age, sulcate-striate nearly to the apex, pallid, tawny or greyish ochraceous, 1–2·5 cm. across; gills crowded, whitish, then brownish-black; spores oblong-elliptical, 10 × 4·5 μ; stem about 2·5 cm. long, 2–4 mm. thick, slightly thickened at the base, minutely downy or pruinose, white, hollow.

Caespitose at the base of cotton-wood stumps. United States.

The species resembles *C. micaceus*, from which it is distinguishable by the floccose-squamose coating of the young pileus, and by its more narrow spores (Peck).

Unfortunately the author has not observed, or at least not recorded, the attachment of the gills, hence the section to which the present species belong is uncertain. It cannot go into the section containing *C. micaceus* on account of its floccose veil.

62. **Coprinus alopecia**, Fries, Epicr. p. 248.

Pileus ovate, then campanulate, obtuse, *sulcate*, *at first covered with adpressed fibrils*, soon glabrous, pale brown or ochraceous, up to 7·5 cm. broad; gills adnexed; spores . . . ? stem at first short, thin, 9–12 cm. long, *densely scaly*, hollow, base thickened.

On trunks of oaks and poplars. Sweden, Germany, France.

Caespitose; margin at first undulately plicate, then split and revolute.

63. **Coprinus Boudieri**, Quél., Bull. Soc. Bot. France, Vol. xxiv, p. 321, pl. 5, f. 4.

Ovoid, then campanulate, *coarsely striate, pale tawny, apex darker*, covered with a fine white pubescence, 1–2 cm. across; gills adnate; spores angularly globose, 10–12 μ; stem 3–4 cm. long, white, *pruinose and pubescent.*

On charcoal-beds. France, Finland.

64. **Coprinus Seymouri**, Peck, 28th Rep. State Mus. N. York, p. 49.

Caespitose, fragile; pileus thin, soon expanded, smooth or sprinkled with small, granular scales, *dark brown*, disc sometimes with a reddish tinge, *strongly striate*, 1·5–2·5 cm. broad; gills reaching the stem; spores broadly ovate, compressed, 6–8 × 5–6 μ; stem 7–10 cm. long, white, smooth or slightly pulverulent, equal, hollow.

On clay soil. United States.

Allied to *C. micaceus*, but thinner, more fragile, darker in colour, and narrower gills.

65. **Coprinus discipes**, Pat., Journ. Botanique, 1889, p. 339.

Pileus thin, convexo-plane, *entirely covered with slender, longitudinal wrinkles*, blackish brown, villosely furfuraceous, 1·5 cm. across; gills adnate; spores 8–10 × 6 μ; stem about 3 cm. long, cylindrical, *springing from a yellow, downy, mycelial disc.*

On horse-dung. Martinique.

66. **Coprinus subcoeruleo-griseus**, Schulzer, Verhandl. Zool. Bot. Gesell. Wien, Band 28, p. 431.

Pileus very delicate, *acutely conical*, then plane, slightly striate, *disc pale yellowish pink*, remainder greyish blue, scattered with minute fugacious scales, 1·5–2 cm. high and broad; gills adnexed; spores 10–13 × 6–8 μ; stem 3–4 cm. long, white, pruinosely floccose, then glabrous, hollow.

On horse-dung; gregarious. Austria.

67. **Coprinus macropus**, B. & Br., Linn. Soc. Journ. (Bot.), Vol. xi, p. 560. (Fig. 41).

Pileus conical, then campanulate, finely striate, *brown, at first covered with evanescent, white flecks*, 6–8 cm. across; gills adnexed, brown; stem up to 15 cm. long, *equal, white, glabrous, rather rooting*, hollow.

On the ground. Ceylon.

68. **Coprinus virgineus**, Peck & Banning, 44th Rep. N. York State Mus. p. 71.

Pileus ovate, campanulate or cylindrical, *pale ochre*, margin thin, torn, floccose; gills adnexed, *forked*; spores black; stem 8–9 cm. long, stout, *flattened, floccose, stuffed.*

Caespitose or gregarious at the roots of trees or about old stumps. United States.

68*. **Coprinus gigasporus**, Massee (sp. nov.) (Figs. 3–5.)

Pileus elliptical, then campanulate, finally upturned, flesh thin, *entirely black*, at first with a sprinkling of white squamules, finely striate up to the disc, 5–6 cm. high; gills free, rather distant from the stem, black; spores elliptical, ends obtuse, black, 28–30 × 14–16 μ; stem 12–17 cm. long, snow-white, equal, hollow.

On dung. Brisbane, Queensland (Bailey, No. 692).

This specimen was referred by Dr. Cooke to *Coprinus picaceus*, from which it differs in the nature of the veil, free gills, distant from the stem, and gigantic spores, which are the largest produced by any member of the Agaricineae.

69. **Coprinus nycthemerus**, Fries, Epicr. p. 251.

Pileus conico-cylindrical, then expanded, *plicate, ribs forked at the margin*, floccosely mealy, then naked, grey, disc tawny, 1·5–2 cm. broad; gills free; stem 5–7 cm. long, white, *glabrous, flaccid, hollow.*

Subcaespitose; on dung and manured ground. Britain, France, Sweden, Hungary, Switzerland, United States.

70. **Coprinus gonophyllus**, Quél., 14th Suppl. Jur. et Vosg., Ann. Sci. Nat. Bord. 1884, pl. 1, f. 2.

Pileus hemispherical, striate, blackish grey, shining, veil caducous, floccose, whitish, about 1·5 cm. broad; gills free, *triangular, margin*

serrate; spores lemon-shaped, 10 μ long; stem about 3 cm. long, glabrous, *slightly striate*, white.

Ground in coal-yards. France.

71. **Coprinus subglobatus**, Berk. & Curt., Proc. Amer. Acad. Arts. & Sci. 1858, p. 118.

Pileus hemispherical, then expanding, almost even, pale brown, covered with a thick, whitish, downy veil, about 4–5 cm. across; gills free, broad, white, then *dusky purple*; spores elliptical, 7–8 μ long; stem white, equal, slightly curved, hollow, smooth, 6–8 cm. high.

On banks. California.

Distinguished among allies by the subglobose form of the pileus when young, and the thick veil.

72. **Coprinus rubecula**, B. & Br., Linn. Soc. Journ. Vol. xi, p. 560. (Fig. 40.)

Pileus *broadly ovate*, then campanulate, white, with a grey tinge, *covered with acute chestnut-coloured scales when young*, the margin becoming naked, very slightly striate, about 1·5 cm. high and wide; gills free; stem 2–3 cm. high, white, smooth, hollow.

On decaying vegetable matter. Ceylon.

73. **Coprinus arcuatus**, Peck, 46th Rep. N. York State Mus. p. 27.

Pileus *broadly ovate or subhemispherical*, soon convex or campanulate, margin striate, white or greyish, darker with age, with small, white tomentose scales, 2·5–5 cm. broad; *gills broad*, free; spores 7·5–9 × 5·5–7·5 μ; stem 2·5–5 cm. long, equal, white, glabrous, hollow.

Solitary or gregarious; on sandy soil recently overrun by fire. United States.

The mycelium binds the sand together into a globular mass. Scales of pileus easily separable, and soon disappear. Cystidia numerous, long.

74. **Coprinus Spraguei**, Berk. & Curt., Ann. Nat. Hist., Oct. 1859, p. 292.

Pileus conical, then campanulate, tomentose, greyish, disc tawny, striate, up to 2 cm. across; gills free, *few and distant*; spores ellip-

tical, slightly curved, 10 × 5 μ; stem 3–5 cm. high, smooth, *pale reddish ochre.*

On the ground. Cuba, United States, Britain.

Distinguished by the coloured stem.

75. **Coprinus Spegazzinii**, Karst., Ryssl., Finl. o. Skand. Hattsv. 1, p. 550.

Pileus very thin, *cylindrical or oval*, then expanding and splitting up to the disc, greyish, at first with a cobweb-like covering and even, soon naked and *grooved*, about 2 cm. high and 3 cm. across; gills free; spores 9–14 × 5–6 μ; stem about 7 cm. long, white, hollow, *thickened below and rooting*, adpressedly silky.

On soil in a plant-pot. Finland.

76. **Coprinus cubensis**, B. & C., Linn. Soc. Journ. (Bot.) Vol. x, p. 293.

Pileus conical, then *conico-campanulate*, covered with superficial, white, floccose scales, grey, even, 2–3·5 cm. across; gills free, *purplish brown*; spores broadly oval or subglobose, 7 × 5–6 μ; *stem 2–3 cm. long*, base thickened.

Growing on logs. Cuba (Wright, No. 79).

Remarkable for the acutely campanulate pileus and the short stem.

77. **Coprinus platypus**, Berk., in Cke., Illustr. pl. 687 B.

Pileus campanulate, white, then yellowish, flocculose, 5 mm. across; gills free; spores 8 × 6 μ; stem 1·5–3 cm. long, slender, *base discoid.*

On palm-stem in a conservatory. England.

Probably an extra-European species, introduced along with plants or soil.

78. **Coprinus rotundosporus**, Peck, 31st Rep. State Mus. N. York, p. 34.

Pileus *campanulate, whitish or pale cinereous*, with a thin floccose, subpersistent tomentum, even, about 2·5 cm. across; gills free; *spores subglobose*, 8–9 μ; stem white, slightly tapering upwards, 5–8 cm. long.

About the roots of trees. United States.

This species is apparently related to *C. niveus*, and is remarkable for its nearly globose spores.

79. **Coprinus narcoticus**, Fries, Epicr. p. 250.

Foetid. Cylindric-clavate, then expanded, greyish white, *hyaline*, striate, *covered at first with white, floccose squamules*, then naked, 1·5–2 cm. across; gills free; spores 11 × 5–6 μ; stem 4–5 cm. long, white, *downy at first*, hollow.

In tufts on dung. Britain, Sweden, France, Germany, Switzerland.

Agrees with *C. muralis* in the strong smell, differs in the hyaline pileus and elliptical spores.

80. **Coprinus muralis**, Allesch. Sued-bayr. Pilze, p. 100.

Pileus membranaceous, smell strong, ammoniacal, cylindrical, then campanulate, striate, white, then grey, covered with white, floccose squamules, 2·5–3·5 cm. high; gills free, narrow; spores subglobose, 6 μ diameter; stem up to 12 cm. long, equal, hollow, *floccosely squamose*, then glabrous, pure white, somewhat shining, base densely fibrous.

On walls, &c. Bavaria.

81. **Coprinus lagopus**, Fries, Epicr. p. 250.

Pileus cylindrical, then campanulate, *coarsely striate up to the brown disc*, remainder whitish, at first covered with white, flocculent down, then naked; 2·5–5 cm. across; gills free; spores 14–16 × 10–12 μ; stem 10–15 cm. long, white, *everywhere covered with white floccose down*, hollow, fragile.

On dung, rotten wood, &c. Britain, Sweden, France, Germany, Holland, Italy, Finland.

Distinguished from *C. narcoticus* by its smaller size and absence of smell, and from *C. fimetarius* in the down on the pileus not being in the form of squarrose scales. *C. lagopides* differs from the present in the tomentum breaking up into scales, and gills very distant from the stem.

82. **Coprinus lagopides**, Karst., Ryssl., Finl. o. Skand. Hattsv. 1, p. 535. (Figs. 20–22.)

Pileus very thin, campanulate, sulcate, *greyish, disc livid*, ornamented with free white scales joined by hairs, 4–7 cm. broad; *gills*

distant from the stem, black; spores 6–8 × 5–6 μ; stem up to 17 cm. high, white, *floccose*, hollow.

On the ground among poplars. Finland.

Allied to *C. lagopus*.

83. **Coprinus macrocephalus**, Berk., Engl. Flora, Vol. v, p. 122.

Pileus *cylindrical*, then campanulate, margin slightly striate, *ashy grey*, *disc brownish*, sprinkled with pale, *pointed scales*, 2 cm. high and broad; gills free; spores 11–13 × 7–8 μ; stem 3–5 cm. long, dingy white, *clothed with white fibrils*, hollow.

Subcaespitose on rotten dung. Britain, France.

Allied to *C. lagopus*, but distinguished by the dark grey pileus, which is coarsely sulcate up to the umbo.

84. **Coprinus tigrinellus**, Boud., Bull. Soc. Bot. Fr. Vol. xxxii, p. 283, pl. 9, f. 3. (Figs. 33–34.)

Pileus *elliptic-oblong*, then campanulate, striate, snow-white at first, margin becoming rosy-grey, pulverulent, *with scattered tawny flecks upwards*, about 1 cm. high and wide; gills free, white, then brownish black; spores broadly elliptical, tawny, 11 × 7 μ; stem about 2 cm. long, white, *base slightly bulbous and often with blackish flecks*.

On leaves of sedges, Iris, and other aquatic plants, growing just above water-level. France.

Closely allied to *C. Friesii*; differing in being covered at first with brown tomentum, which breaks up into flecks on pileus and base of stem.

85. **Coprinus Friesii**, Quélet, Champ. Jura, p. 129, pl. 23, fig. 5. (Figs. 37–38.)

Pileus elliptic-oblong, then expanding, floccosely pulverulent, delicately striate, white or apex tinged yellow, margin becoming rosy-grey, 1–2 cm. high and across; gills free, *reddish*, *then black*; spores angularly subglobose, about 10 μ long; stem 2 cm. long, slender, white, *pulverulent*, base rather swollen and floccose.

On dead grass and other leaves. France, Holland, Germany, Belgium, Finland.

Differs from *C. tigrinellus*, which also grows on grass, &c., in not being covered when young with a brown veil.

86. **Coprinus cupulatus**, E. Jacob., Mitteil. Brandenb. p. xxxi.

Pileus subcampanulate, covered at first with a floccose, greyish white veil, then pubescent, *sulcate*, *apex depressed*, greyish yellow, about 4–5 mm. high; gills free; spores 7–8 × 6–7 μ; stem 5–7 mm. high, white, downy, *striate*, *base swollen into a small, slightly strigose bulb.*

On dead twigs. Germany.

Allied to *C. Friesii*, *C. tigrinellus*, and *C. Quéletii*.

87. **Coprinus fimetarius**, Fries, Epicr. p. 245.

Pileus clavate, then conico-expanded, soon splitting, *coarsely grooved*, greyish, disc even and brownish, *at first covered everywhere with squarrose, floccose scales*, then naked, 2·5–5 cm. across; gills free; spores 12–14 × 7–8 μ; stem 10–15 cm. long, white, *squamulose*, hollow, base thickened and solid.

On manure-heaps, &c., solitary or most frequently clustered, soon becoming revolute and deliquescing.

Britain, Austria, Sweden, France, Switzerland, Germany, Siberia, Holland, Spain, Portugal, Italy, Russia, Belgium, Finland, Hungary, Australia, New Zealand.

Var. **pullatus**, Fries.

Pileus with adpressed squamules, soon naked, brown, then blackish, stem soon smooth. Stature of typical form.

Var. **cinereus**, Fries. (= *Agaricus cinereus*, Schaeff., Icon. tab. 100.)

Pileus floccosely mealy, then naked, grey; stem rootless, subequal, hollow to the base, often twisted. Size of typical form.

Var. **macrorhiza**, Fries. (Figs. 1–2).

Pileus at first with feathery squamules, stem short, rooting. Paler and smaller than the typical form.

The above varieties grow on dung or rich soil, and appear good varieties in their extreme forms, but blend into the typical form and into each other.

88. **Coprinus Quéletii**, Schulzer, Hedw. 1885, p. 137.

Pileus ellipsoid-conical, then expanded, *deeply sulcate*, whitish; apex glabrous, tinged cinnamon, sprinkled with fugacious flecks, about 2·5 cm. broad; gills free; spores elliptic-oblong, 10–12 × 4–6 μ;

stem up to 4 cm. long, *ventricose below*, white, flocculose, then glabrous and silky; *rooting, fibrils up to 2 cm. long, brown.*

On soil in plant-pots. Austria.

89. **Coprinus laxus**, Bresad. & Schulz., Hedw. 1885, p. 136.

Pileus *parabolic, then semiglobose*, grey, disc yellowish cinnamon, *granuloso-floccose*, 1–1·2 cm. broad; gills free, 2 mm. broad; spores irregularly elliptical, black, 6–8 × 4–5 μ; stem 2·5–6·5 cm. long, white, *tinged brown at the slightly thickened base, with fugacious flecks, especially upwards.*

In pastures. Austria.

The stem bends over as it dries.

90. **Coprinus Albertinii**, Karst., Hattsv. 1, p. 535.

Pileus campanulate, *sulcate, greyish white*, disc brownish, clothed with a delicate network of fibrils, 3–4 cm. broad; gills free, becoming distant from the stem; spores elliptical, opaque, 10–12 × 6 μ; stem up to 7 cm. long, *silky flocculose*, white.

Grassy places. Finland.

91. **Coprinus plumbeus**, Peck, 29th Rep. State Mus. N. York, p. 42.

Pileus thin, fragile, campanulate, *deeply striate nearly to the apex*, leaden grey, tawny on the small disc, *sprinkled with tawny-cinereous hairs*, 2·5–4 cm. across; gills free, narrow; spores elliptical, 10 × 6 μ; stem 8–12 cm. high, white, *floccose*, hollow, slightly tapering upwards.

Paths in woods. United States.

92. **Coprinus Strossmayeri**, Schulzer, Verhandl. Zool. Bot. Gesell. Wien, Band 28, p. 430.

Pileus *digitaliform*, then conico-campanulate, whitish, then grey, apex darker, finally pale ochraceous, covered with superficial, seceding, *squarrose scales*, not striate, 5–8 cm. broad; gills free; spores 7–9 μ long; stem 4–16 cm. long, straight or ascending, white, delicately *pruinose*, hollow, springing from a blackish brown, compact, branching mycelium.

Subcaespitose at roots of trees. Austria.

93. **Coprinus tuberosus**, Quél., Bull. Soc. Bot. France, Vol. xxiv, p. 289, pl. 3, fig. 2. (Figs. 26–30.)

Pileus elliptical, then campanulate, finely striate, white, then greyish, *veil formed of hyaline vesicles*, 3–5 mm. high; gills blackish violet; spores elliptical, about 12 μ long; stem 2–4 cm. long, very slender, rather flexuous, white, downy, *springing from a small, black sclerotium*.

On dung or decaying vegetable matter. France, England.

Somewhat resembling *C. niveus*, but smaller, and differing in springing from a sclerotium.

94. **Coprinus cineratus**, Quél., Bull. Soc. Bot. Fr. Vol. xxiii, p. 329. pl. 2, f. 7.

Pileus cylindrical, then campanulate, striate, enveloped at first in a white, then greyish violet, *volva formed of hyaline vesicles*, dusky grey, 1–2 cm. high and broad; gills free, close to the stem; spores elliptical, 10 × 5 μ; stem 4–6 cm. high, white, base slightly swollen and sheathed by the remains of the volva.

Caespitose; on dung, &c. France.

95. **Coprinus filiformis**, B. & Br., Ann. & Mag. Nat. Hist., Ser. 3, Vol. vii, p. 7, pl. 15, fig. 8.

Pileus *cylindrical*, grey, covered with white mealy particles, striate, 1–2 mm. high; gills linear; spores subglobose, 5 × 4 μ; stem 1–1·5 mm. high, extremely slender, hyaline, *sprinkled with short, delicate hairs*.

On the ground in woods. England.

This minute species is not larger than *Mucor caninus* (B. and Br.).

96. **Coprinus luxoviensis**, Mont., Ann. Sci. Nat., Ser. 4, Vol. ix, p. 161.

Pileus very delicate, *ovoid*, then campanulate, delicately striate, at first covered with white meal, then naked and grey, *disc tawny*; gills semilanceolate, distant; *stem at first with spreading fibrils*, soon naked, white, pellucid, 2·5 cm. long, springing from a spreading mycelium.

On walls. France.

97. **Coprinus caducus**, Harz., Bot. Centralb. xxvii, p. 416.

Pileus oval, then cylindrical, striate, grey in the cylindrical state, then blackish brown, disk dark grey, *at first covered with a dense snow-white powder*, which becomes grey, 8–11 mm. high; gills springing from a collar; spores elliptical, 9–10 × 6–7 μ; stem variable in length, 2–12 cm. long, *greyish brown above*, base whitish.

In shaft of coal mine. Bavaria.

98. **Coprinus Britzelmayri**, Sacc. & Cub., Syll. v, No. 4447 (*Coprinus macrosporus*, Britz., Melanosp. p. 183, f. 129; not of Peck).

Pileus and stem covered with snow-white meal; pileus 2 cm. and more broad; gills adnexed, greyish black; spores elliptical, 20 × 10–12 μ; stem up to 7 cm. high by 5 mm. thick.

In meadows. Bavaria.

99. **Coprinus albulus**, Quél., Assoc. France, Congrès de Rouen, 1883, p. 4, tab. 6, f. 11.

Pileus almost hemispherical, *pellucid*, striate, minutely pulverulent, 5 mm. broad; gills arcuately adnate; spores ovoid, 20 μ long; stem 2 cm. long, filiform, *pulverulent, with a slender rooting base.*

On fallen grass-stems (*Triticum*). France.

Resembling *Mycena tenerrima* in habit.

100. **Coprinus semilanatus**, Peck, 24th Rep. State Mus. N. York, p. 71.

Pileus broadly conical, then expanded, *atomate*, finely striate, pale greyish brown, 1·5–2·5 cm. broad; gills free; spores 13 μ long; stem 10–15 cm. long, white *lower half dotted with loose, cottony flecks*, smooth or slightly mealy above, hollow.

On rich ground and dung. United States.

Very fragile. Allied to *C. cöpertus*.

101. **Coprinus divergens**, Britz., Melan., p. 182, fig. 64.

Pileus parabolic, *brownish at first*, margin paler, becoming dark grey, *deeply striate*, hoary at first, 1·5 cm. broad, gills blackish grey, spores elliptical, ends acute, 10–11 × 6–7 μ; stem up to 4 cm. high, *pellucid*, whitish.

In pastures. Bavaria.

Intermediate between *C. tomentosus* and *C. niveus* (Britzelmayr).

SECTION V.

102. **Coprinus micaceus**, Fries, Epicr. p. 247.

Pileus *oval at first*, then campanulate, *margin plicate and irregular*, striate, tawny ochraceous, at first covered with glistening micaceous particles, *soon naked and becoming sulcate*, 3–6 cm. across; gills adnexed; spores 7–8 × 4–5 μ; stem 5–8 cm. long, *white*, *silky*, hollow.

Usually densely clustered, at the base of old stumps, posts, &c. Britain, France, Finland, Germany, Hungary, Siberia, Austria, Sweden, Holland, Spain, Portugal, Italy, Russia, Belgium, Switzerland, United States, Cape of Good Hope, Victoria.

At first densely covered with colourless micaceous particles; soon naked in rainy weather, when the pileus often becomes brownish. See remarks under *C. truncorum*.

103. **Coprinus marcescens**, Karst., Ryssl. Hattsv. 1, p. 537.

Pileus campanulate, sulcate, *whitish at first*, soon dingy ochraceous, *at length pale sooty-grey*, disc brownish yellow, micaceous, sulcate, 3–4 cm. broad; gills adnexed; spores obliquely elliptical, 6–9 × 4–6 μ; stem about 9 cm. long, white, silky but even.

On the ground. Finland.

Allied to *C. micaceus*, but distinguished by the pileus becoming sooty-grey.

104. **Coprinus aratus**, B. & Br., Brit. Fung., No. 927, Ann. Nat. Hist. (1861); emended, No. 1956.

Pileus *narrowly elliptical*, then campanulate, *umber*, *deeply grooved up to the disc*, sprinkled with large micaceous particles, 5–8 cm. across; gills slightly adnexed, broadest behind; spores 15 × 10–11 μ; stem 10–15 cm. long, snow-white, silky, hollow.

In hollow trees, on the ground, &c. Britain.

Berkeley and Broome at first described the present species as having free gills, and afterwards this was corrected to 'gills at first attached, but soon separating from the stem, but still connected at the base as if by a slight collar.'

105. **Coprinus stercorarius**, Fries, Epicr. p. 251.

Pileus ovate, then campanulate, margin striate, *densely covered with white glistening meal*, 2·5–3 cm. high and broad; gills adnexed; spores 14–15 × 8 μ; stem 7–12 cm. long, white, *minutely mealy at first*, hollow.

On dung, manured ground, &c. Britain, Sweden, Belgium, Germany, France, Finland, Holland, Italy, Switzerland, Austria, Victoria, Queensland, Tasmania, New Zealand, United States.

Superficially resembles *C. niveus*, but the latter has the pileus covered with white, floccose down, not meal, and is altogether smaller.

C. albus, Quél., differs in having the disc more or less yellow, pileus sulcate, as is also the upper part of the stem.

106. **Coprinus radians**, Fries, Epicr. p. 248. (Figs. 6–8.)

Pileus ovate, then campanulate, glistening with micaceous particles, margin striate, *disc granuloso-squamulose*, tawny ochre, becoming pale, 2·5–5 cm. across; gills slightly adnexed; spores 7 × 4 μ; stem 3–5 cm. long, white, smooth, hollow, *base with dense, radiating strands of mycelium*.

On damp plastered walls. Britain, France, Sweden, Belgium, Finland.

Stem incurved from growing on a vertical surface, pileus becoming discoid.

107. **Coprinus truncorum**, Fries, Epicr. p. 248.

Pileus *globose at first*, then campanulate, densely covered with micaceous meal, soon naked, then tawny ochraceous, striate, *not becoming sulcate*, 2–4 cm. across; *gills free, rosy*, then black; spores 12–14 × 6 μ; stem 7–10 cm. long, slender, white, glabrous, hollow.

On rotten willows, &c. Sweden, Britain, Holland, Germany, France, Switzerland, Belgium, Austria, Cape of Good Hope, Australia.

Allied to *C. micaceus*, but distinguished by the pileus being globose at first, never sulcate, and the free rosy gills.

108. **Coprinus inamoenus**, Karst., Grevillea, Vol. vii, p. 63.

Foetid. Pileus subcylindrical, then expanded, *blackish*, densely covered with white micaceous scurf, even, 2–5 cm. broad; *gills*

attached to a collar remote from the stem; spores elliptical, 7–11 × 4–6 μ; stem 4–7 cm. long, often flexuous, hyaline-white, at first downy; several stems springing at intervals from a prostrate mycelium.

On heaps of rotten leaves. Finland.

109. **Coprinus intermedius**, Penzig, Ozonium et Copr. p. 140, pl. 3 and 4.

Pileus cylindrical, then campanulate, pallid, even, at first *densely covered towards the apex with reddish micaceous scurf*, 3–3·5 cm. broad; gills free, pinkish at first; spores elliptical, 7·5–9 × 5 μ; stem glabrous, *tinged pink*, 9–11 cm. long.

On damp wood, and on soil in flower-pots in hothouse. Italy.

Intermediate between *C. stercorarius* and *C. coöpertus*. Differs from former in cylindrical—not ovate—pileus, covered with rufescent —not white—micaceous scurf; from the latter in the glabrous stem.

110. **Coprinus frustulosum**, Sacc., Myc. Ven. Spec. p. 35, t. 6, f. 10–14, from Atti della Soc. Ven.-Trent. Vol. ii.

Pileus ovate, then campanulate, rather acute, even, covered with reddish micaceous meal, up to 1 cm. high; gills free; spores 8 × 6 μ; stem 1·5 cm. high, conical, then cylindrical, *white*, hollow, smooth.

On fragments of roots and twigs. Italy.

Section VI.

111. **Coprinus conditus**, Godey, in Gill. Champ. Fr. Hymen. p. 612.

Pileus *globose*, then ovoid, striate, white or tinged yellow, furfuraceous or slightly micaceous; gills adnate; stem 20–25 mm. high when deliquescing, shining, *furfuraceous*.

In the interstices of cow-dung. France.

112. **Coprinus nebulosus**, Zoll., Flora, 1847, p. 305.

White; *pellucid*; pileus with the disc granular when young, then splitting into greyish ridges, 1–1·5 cm. across; gills linear; stem about 6 cm. long, *bulbous at the base*.

On buffalo-dung. Java.

113. **Coprinus stellaris**, Quél., Bull. Soc. Bot. France, Vol. xxiv, p. 322, pl. 5, fig. 6.

Pileus ovoid, then campanulate, striate, snow-white then greyish, *crowned with minute pellucid vesicles*, 1–2 mm. across; gills adnate; spores elliptical, about 8 μ long, stem 1–2 cm. long, filiform, hyaline, *velvety* with slender white hairs.

On dung of man, fox, &c., in caves. France.

114. **Coprinus coöpertus**, Fries, Epicr. p. 252.

Pileus conico-campanulate, striate, lurid, yellowish grey when dry, densely covered with micaceous particles, up to 2·5 cm. across; *gills adnate, broad*; stem 3–5 cm. long, pallid, *apex with delicate white flecks*.

On dung. Sweden, France, Italy, Hungary.

Somewhat caespitose; very fragile.

115. **Coprinus pseudo-plicatilis**, Voglino, Rich. Anal. Agaric. p. 42, t. 50, f. 4.

Pileus campanulate, umbonate, soon expanded and sulcate, yellowish grey, *umbo yellowish*, furfuraceous, 8–10 mm. broad; gills adnate; spores 6–8 × 3, obliquely elliptical; stem 2·5–4 cm. high, slender, *woolly, white, thickened and floccose downwards*.

On wood in a hothouse. Italy.

Springing from an Ozonium-like weft of ochraceous hyphae. Differs from *C. plicatilis* and *C. sulcato-crenatus* in the downy stem and absence of collar at apex of stem.

116. **Coprinus aquatilis**, Peck, 27th Rep. N. York State Mus. p. 96, pl. 1, figs. 26–28.

Pileus campanulate, sulcate-plicate almost to the apex, scurfy, *yellowish brown*, 1·5–2 cm. across; gills reaching the stem, brownish, then black; spores 13 × 8 μ; stem 5–7 cm. high, slender, equal, hollow, *whitish, scurfy*.

On sticks or twigs partly submerged or lying in wet mossy places. United States.

The young plant is more yellow than the mature one. The species is related to *C. silvaticus*.

117. **Coprinus velox**, Godey, in Gill. Champ. Fr. Hym. p. 614, with fig.

Pileus obovate, striate, then grooved, scurfy between the ribs, disc also greyish and scurfy, 3–4 mm. across; gills close to the stem; stem 1·5–3 cm. long, *covered with delicate white floccose down, base with radiating fibrils.*

On cow-dung. France.

118. **Coprinus ephemerus**, Fries, Epicr. p. 252.

Pileus very delicate, ovate, then campanulate, sulcate, slightly scurfy at first, *disc elevated, even, rufescent*, 1–2 cm. across; gills slightly adnexed; spores 16–17 × 9–10 μ; stem 3–6 cm. long; *glabrous, pellucid*, whitish, hollow.

On dung-hills, manured ground, &c. Britain, France, Denmark, Germany, Holland, Spain, Portugal, Sweden, Italy, Russia, Belgium, Finland, Hungary, Switzerland, United States, Queensland, New Zealand, South Africa.

Very slightly furfuraceous at first, soon naked.

119. **Coprinus mycenopsis**, Karsten, Symb. Myc. Fenn. viii, p. 8.

Pileus campanulate, then expanded, *sulcate, sooty-grey*, the livid *disc prominent*, scurfy at first, soon naked, 3–7 cm. across; gills adnate, *purple, then brown*; spores 7–8 × 4 μ; stem up to 14 cm. long, glabrous (apex very slightly flocculose), *striate upwards*, white, hollow.

In meadows. Finland.

120. **Coprinus Berkeleyi**, Montag., Syll. Pl. Crypt. No. 409, p. 131.

Pileus cylindrical, ovate, then campanulate, delicately striate, furfuraceous, yellowish or greyish brown, then blackish, 3·5 cm. high, 5 cm. broad; gills adnexed, very narrow; stem up to 15 cm. long, *cartilaginous, variegated with yellowish green and rufous*, hollow, glabrous, base thickened.

On rotten wood. United States.

Differs from *C. stenophyllus* in colour, absence of scales on pileus, and in having margin of pileus entire, and from *C. deliquescens* in the pileus being delicately striate and the adnexed gills.

120*. **Coprinus australiensis**, Mass. (sp. nov.). (Figs. 31–32.)

Pileus elliptical, soon becoming plane, finally upturned and umbonate, flesh very thin except at the disc, almost glabrous, pale tan, becoming greyish towards the margin, disc darker (when dry), striate, 5–7 cm. across when plane; gills *free*, *broad*, black, margin whitish, not readily deliquescing; spores *lemon-shaped*, *black*, 17–18 × 10–12 μ; stem 10–14 cm. long, almost equal, whitish.

On dung. Brisbane, Queensland (Bailey, No. 691).

A very distinct and beautiful species, at first referred to *C. deliquescens*, from which it differs in the much larger spores, and other points.

121. **Coprinus cothurnatus**, Godey, in Gillet's Champ. Fr. Hymen. p. 605, with fig.

Pileus *conico-campanulate*, finally expanded and umbonate, densely furfuraceous, dingy white, reddish, flesh-coloured, or yellowish, 2–3 cm. across; gills free; spores elliptical; stem 3–5 cm. long, white, *squamulose below*.

On cow-dung. France, Britain.

The strigose squamules surrounding the base of the stem represent a completely disintegrated volva.

122. **Coprinus evanidus**, Godey, in Gill. Champ. Fr. Hym. p. 614, with a fig.

Pileus *obovate*, then campanulate, striate, whitish, slightly furfuraceous, *disc prominent*, *tinged brown*, 3–4 mm. across; gills free, distant; spores elliptical; stem 1·5–2·5 cm. long, pellucid, white, covered with delicate white down.

In the interstices of cow-dung. France.

123. **Coprinus sociatus**, Fries, Epicr. p. 252.

Pileus ovate, then campanulate, plicate, subsquamulose or furfuraceous, pallid, *disc umber*, *at length depressed*, about 2 cm. across; gills narrowed behind, *attached to a collar*, but not remote from the stem, blackish; stem 4–5 cm. long, white, glabrous, not pellucid.

Damp ground in gardens, &c. Denmark, Sweden, Britain, France, Germany, Holland, Belgium, Switzerland.

Distinguished from *C. plicatilis* by the narrow collar, hence the gills are near to the stem, much narrowed behind, and black.

Fries describes a variety—Monogr. 1, p. 468—having the collar obsolete, and the gills touching the stem.

124. **Coprinus sulcato-crenatus**, Steinhaus, in Sacc., Syll. v, No. 4492.

Pileus cylindrical, then campanulate, soon plane, sulcato-crenate, almost glabrous, yellow; *disc brown, even, becoming depressed*; gills remote from the stem, distant; spores laterally compressed, obtusely triangular; stem filiform, glabrous, *yellow, apex brown.*

On horse-dung. Poland.

Allied to *C. plicatilis*, differing in the yellow pileus and stem.

125. **Coprinus Patouillardii**, Quél., in Pat., Tab. Anal. Fung. p. 107, fig. 240.

Pileus *conico-campanulate*, then plane, coarsely striate up to the disc, ashy-grey, *disc yellowish and rough with minute reddish granules or flecks*, 1–2 cm. across; gills free, attached to a collar and distant from the stem; spores angularly globose, 6–8 μ diameter; stem 4–7 cm. long, white, glabrous, fragile.

In clusters on remains of decayed grapes. France.

Distinguished from its close ally, *C. nycthemerus*, by the pure white stem and differently shaped spores. *C. velaris* differs in the glabrous pileus.

126. **Coprinus papillatus**, Fries, Epicr. p. 248.

Pileus elliptical, then campanulate, becoming plane or upturned, but *the disc remaining prominent and rough with minute warts*, striate, covered with a greyish scurf, disc darker, up to 1 cm. across; gills free; spores 15 × 7 μ; stem about 2·5 cm. long, white, *hyaline*, hollow.

On the ground and on dung. Britain, Sweden, Germany, France, Russia, Belgium, Switzerland.

127. **Coprinus Wrightii**, Berk. & Curt., Ann. & Mag. Nat. Hist., 1859, p. 10.

Pileus *oval*, then plane, striate, glaucous, *scurfy, with small brown flecks*, 1–1·5 cm. across; gills free; spores obliquely elliptical,

9–10 × 6 μ; stem 5 cm. long, slender, whitish, smooth, pellucid, hollow.

On bits of grass, and in a flower-pot. United States.

128. **Coprinus affinis**, Karst., Ryssl., Finl. o. Skand. Hattsv. 1, p. 536.

Pileus very delicate, *conico-cylindrical*, then expanded, greyish white, *disc pale rufous*, plicate, scurfy, scarcely 1 cm. broad; gills free, narrow; spores 6–8 × 5–7 μ; stem about 3 cm. long, slender, flaccid, glabrous, *pallid*.

On naked ground and on wood. Finland.

129. **Coprinus curtus**, Kalchbr., Grev. Vol. ix, p. 133.

Pileus *cylindrical*, then ovate campanulate, at *first covered with rust-coloured scurf*, then grey, sulcate, 6–8 mm. high; gills free; *stem 1–1·5 cm. long*, glabrous, hollow, base with white down.

On dung. South Africa.

Allied to *C. plicatilis*, but smaller and scurfy.

130. **Coprinus radiatus**, Fries, Epicr. p. 251.

Pileus cylindrical, then campanulate; *soon plane, sulcate and splitting*, at first with greyish scurf, yellowish, disc tawny, 3–4 *mm. across*; gills free; spores 7–8 × 5 μ; stem 1–2 cm. high, hyaline, nearly glabrous, whitish.

On horse-dung, often on the underside or in the interstices. Britain, France, Germany, Holland, Spain, Portugal, Sweden, Italy, Russia, Belgium, Finland, Switzerland, Cape of Good Hope, United States.

Distinguished by its minute size, and the plane, radiately fissured pileus.

131. **Coprinus lanatus**, Bong., in Weinm. Ross. p. 279.

Pileus soon campanulate, plicately striate, *greyish rufous, disc darker*, 1·5–2·5 cm. high; gills free, *purplish brown*; stem *slightly bulbous*, glabrous, white, 2·5–5 cm. long.

Under hedges, &c. Russia.

Solitary; fugacious.

132. **Coprinus tardus**, Karst., Symb. Ad. Myc. Fenn. vi, p. 20.

Pileus ovoid, then campanulate, *coarsely striate*, bay, then paler, glabrous, 2·5–5 cm. high and broad; gills adnate; spores angularly

ovoid, 12–18 × 7–9 μ; stem 6–10 cm. high, sometimes *slightly flexuous*, equal, white, pulverulent.

Caespitose; on naked ground. Finland, England, Hungary.

Smell none; differing from *C. deliquescens* in the smooth—not papillose—disc, and in the pileus not becoming upturned, and adnate, crowded gills.

133. **Coprinus mutatinus**, Mont., Cent. vii, p. 30.

Entirely grey; pileus very delicate, at first conico-convex, soon plane, very glabrous, *slightly striate*, *margin crenulate*; gills slightly adnated, very narrow; stem puberulous, fistulose.

On dung. Brazil.

134. **Coprinus sclerotigenus**, Ellis & Everh., The Microscope, 1890, p. 129, with fig. (Figs. 26–28.)

Pileus ovoid or ovoid-oblong, then campanulate, *blackish brown*, apex tinged whitish, about 1 cm. high and broad; gills adnexed; spores obliquely elliptical, 8–10 × 5–6 μ; stem 2·5–10 cm. high, slender, subequal, usually straight upwards and more or less flexuous below, where it is also downy, *springing from an irregularly subglobose, rugulose, sclerotium*, which is black externally, inside white.

On sheep's dung. United States.

135. **Coprinus auricomis**, Pat., Tab. Anal. Fung. p. 200, fig. 453. (Figs. 15–19.)

Pileus elliptic-oblong, then campanulate, finely striate, pale *greyish red, disc darker*, glabrous, 1·5–2 cm. high and broad; gills adnate; spores ochraceous brown, elliptical; stem 5–8 cm. high, *white*, glabrous.

Clustered, on rotten wood. France.

Young plants enveloped in a golden-yellow *Ozonium*, some of the filaments remaining on the pileus and base of stem at maturity.

136. **Coprinus pachyterus**, B. & Br., Linn. Soc. Journ. (Bot.), Vol. xi, p. 561.

Pileus *persistently campanulate*, plicato-sulcate, smooth, 5 *cm. across*; *gills arcuate*, adnexed; spores elliptical, 14–15 × 7–8 μ; stem 6–8 cm. long, white, smooth.

On the ground. Ceylon.

Allied to *C. plicatilis*, but larger.

137. Coprinus congregatus, Fries, Epicr. p. 249.

Pileus cylindrical, then campanulate, margin slightly striate, glabrous, *viscid, ochraceous*, 1·5–2 cm. high; gills slightly adnexed; stem 2–3 cm. high, smooth, hollow, white.

On the ground, also in hot-houses. Sweden, Britain, France, Germany, Spain, Portugal, Belgium, Finland, Hungary.

Distinguished by the entirely ochraceous, glabrous, viscid pileus. Densely tufted.

138. Coprinus silvaticus, Peck, 24th Rep. N. York State Mus. p. 71, pl. 4, figs. 10–14.

Pileus *convex*, striate half-way up from the margin, *dark brown, the furrows paler*, 1·5–2 cm. across; gills attached to the stem, brownish; spores gibbous-ovate, 12–13 μ long; stem 5 cm. high, fragile, hollow, smooth, slender, white.

On the ground in woods. United States.

Allied to *C. plicatilis* and *C. ephemeris*.

139. Coprinus alternatus, Fries, Epicr. p. 248.

Pileus hemispherical, then discoid, *even*, quite glabrous, *chalk-white, disc pale umber*, 3 cm. broad; gills adnate; spores 10 × 6–7 μ; stem whitish, smooth, hollow, 7–10 cm. long.

In small clusters on the ground. Britain, Denmark.

140. Coprinus angulatus, Peck, 26th Rep. N. York State Mus. p. 60.

Pileus *hemispherical*, plicate-sulcate, disc smooth, 1·5–2·5 cm. across; gills reaching the stem, white, then blackish; spores compressed, angular, subovate, 10 × 9 μ; stem equal, smooth, whitish, 3–5 cm. long.

In woods. United States.

141. Coprinus digitalis, Fries, Epicr. p. 249.

Pileus ovate, then campanulate, *whitish, disc darker*, quite glabrous, *striate up to the disc*, 2·5 cm. high and broad; gills slightly adnexed; stem 3–10 cm. long, equal, rather *flexuous*, glabrous, white.

On the ground in woods. Sweden, Denmark, Britain, France, Germany, Holland, Italy, Finland.

Tufted; fragile. When mature the pileus is livid-olive or yellowish grey, and the gills appear to be adnate.

142. **Coprinus hortensis**, Mont., Syll. Pl. Crypt. No. 406, p. 131.

Pileus very delicate, *silvery-grey*, convex, then campanulate, at length expanded, glabrous, *finely striate up to the even disc*, 3·5 cm. broad; gills slightly adnexed, very narrow; stem 4 cm. long, even, glabrous.

On the ground. Cayenne.

143. **Coprinus diaphanus**, Quél., Bull. Soc. Bot. France, Vol. xxiv, pl. 5, fig. 7.

Every part translucent and glabrous; pileus grooved, margin crenulate, *silvery with a central tawny spot*, 6–8 mm. broad; gills adnate; spores elliptical, 12 μ long; stem capillary, hyaline, glabrous, 2–3 cm. long.

Grassy places in woods, &c. France.

Distinguished from small specimens of *C. plicatilis* by the adnate gills.

144. **Coprinus sceptrum**, Fries, Epicr. p. 253.

Pileus campanulate, *papillately umbonate*, deeply sulcate, *pellucid*, tinged grey, 5–7 mm. broad; gills adnate to a collar; spores black; stem 2·5–5 cm. long, *pellucid*.

Rich ground among grass. Germany, France, Sweden.

145. **Coprinus erythrocephalus**, Fries, Hym. Eur. p. 327.

Pileus cylindrical, then campanulate, silky and shining, margin very finely striate, *reddish vermilion*, becoming grey, about 1 cm. high and broad; gills slightly adnexed; stem 2–3 cm. high, red, paler than the pileus.

Caespitose; on soil mixed with powdered gypsum. France.

Readily distinguished by the red pileus and stem.

146. **Coprinus Godeyi**, Gillet, Champ. Fr. Hym. p. 611, with fig.

Pileus ovoid-globose, distantly grooved, very glabrous, pellucid, *disc ochraceous, grey between the ribs*, 3–4 mm. diameter; gills free; spores elliptical; stem about 2 cm. long, pellucid, *sprinkled with white flecks below*.

On soil in plant-pots. France.

147. **Coprinus semistriatus**, Pat., Tab. Anal. Fung. p. 194, fig. 435. (Figs. 9–12.)

Pileus ovate, then campanulate, glabrous, disc even, yellowish, from margin up to disc grey and striate; about 1 cm. high; *gills attached to a collar*; spores almost circular, compressed, 12–14 × 3 μ; stem 1–2 cm. long, white, *pruinose*, base pilose.

Tufted or scattered; on manured ground, &c. France.

148. **Coprinus consobrinus**, Mont., Cent. vii, p. 30.

White; pileus *pellucid*, centre sometimes tinged yellow, *smooth, shining*, striate, margin denticulate; gills free and *distant* from the stem, very narrow; stem tall, *floccosely squamulose.*

Solitary or gregarious. Brazil.

149. **Coprinus plicatilis**, Fries, Epicr. p. 252. (Figs. 23–25.)

Pileus very delicate, cylindric-ovate, then campanulate, soon plane, *coarsely grooved*, glabrous, *pale brown, then greyish, disc broad, even, at length depressed, darker*, 1–2 cm. across; gills free, *attached to a collar*, distant from the stem; spores 11–13 × 8–9 μ; stem 5–8 cm. long, white, smooth, hollow.

On the ground among grass, &c. Switzerland, Britain, Sweden, Italy, Russia, Belgium, Finland, Hungary, Denmark, Germany, France, Holland, United States, South Africa, E. Tropical Africa, Ceylon, Queensland, New Zealand, Behring Straits, India, Japan.

There is a trace of scurf, especially in the grooves of the pileus when young.

150. **Coprinis mirabilis**, Mont., Syll. Pl. Crypt. No. 407, p. 131.

Pileus very delicate, soon plane and radiately sulcate, *margin crenulate, white*; gills free; spores *globose*; stem slender, hollow, white.

On the ground under trees. Cayenne.

151. **Coprinus deliquescens**, Fries, Epicr. p. 249.

Pileus ovate, then campanulate, finally expanded, *livid grey; disc rufescent, papillose, otherwise glabrous*, 3–7 *cm. across*, often rather wavy; gills free; spores 8 × 5 μ; stem 7–10 cm. long, white, glabrous, hollow.

On trunks, stumps, heaps of leaves, &c. Holland, Spain, Portugal, Russia, Belgium, Finland, Britain, Sweden, France, Germany, Hungary, Switzerland, Queensland.

Slightly tufted. Differs from *C. atramentarius* in the free gills, distant from the stem, and in being altogether more slender.

152. **Coprinus flosculus**, Berk., Flor. Antart. p. 448, tab. clxii, fig. 2.

Pileus ovate, sulcate, *apex depressed*, glabrous, greyish, *margin crenulate*, 3 mm. high, 2 mm. broad, gills free; spores ovate; stem 4 mm. high.

On dung. Berkeley Sound, Falkland Island.

It resembles in habit *C. Hendersonii*, Fr., but differs in the absence of a ring.

153. **Coprinus miser**, Karsten, Ryssl., Finl. o. Skand. Hattsv. 2, p. 236.

Pileus *subglobose*, then expanded, pellucid, tinged grey, plicate, glabrous, 1–2 mm. broad; gills distant from stem, *few in number*; spores broadly elliptical, 7–9 × 6–8 μ; stem 1–2 cm. long, slender, hyaline, glabrous.

On horse-dung. Finland.

154. **Coprinus eburneus**, Quél., Assoc. France, 1883, p. 4, tab. vi, fig. 10.

Entirely white, shining; elliptic-campanulate, firm, striate, rarely with a few scattered flecks, 3–4 cm. broad; gills free; spores ovate, 14 μ long; stem firm, glabrous.

On the ground in upland regions. France.

Allied to *C. exstinctorius*; resembling *Psathyra gyroflexa* in habit.

155. **Coprinus hemerobius**, Fries, Episc. p. 253.

Pileus ovate, then campanulate, coarsely grooved, disc even, bay, not depressed, 1·5–2·5 cm. across; *gills attached to a very slightly developed collar*; stem 5–8 cm. high, very fragile, *pallid*; spores elliptical, 10–12 × 7 μ.

Among grass on roadsides, &c. Sweden, Britain, Germany, Holland, Denmark, France, Italy, Finland.

Differs from *C. velaris* in the imperfect collar at apex of stem, and in the pileus being ovate when young. *C. plicatilis* differs in having the disc depressed at maturity.

156. **Coprinus rapidus**, Fries, Epicr. p. 253.

Pileus cylindrical, then plane, coarsely grooved, *lurid or pale drab*, always glabrous, often slightly undulated, 1·5–2·5 cm. across; gills free but close to the stem, *brown*; stem 4–5 cm. high, white, glabrous.

On the ground near dwellings, &c. Sweden, France, Germany.

Deliquescing rapidly after expansion.

157. **Coprinus modestus**, Berk. & Curt., Proc. Amer. Acad. Arts & Sci. 1858, p. 118.

Pileus very delicate, sulcate, glabrous, *pallid purple*; gills linear, free, black; stem slender.

On decayed wood. Bonin Islands.

There is no specimen in Berkeley's herbarium, hence the original meagre description cannot be supplemented.

Species belonging to the last division with a glabrous pileus, but cannot be arranged in the series, owing to absence of information respecting the attachment of the gills.—47–54.

158. **Coprinus sororius**, Karsten, Symb. Myc. Fenn. viii, p. 9.

Pileus naked (from the first?), about 2 cm. broad; gills white or greyish, then black; spores 9–12 × 6–7 μ; stem 5–6 cm. long, even, obsoletely *flocculose*, hyaline-white, pellucid.

In meadows. Finland.

Similar to *C. mycenopsis*, but smaller (Karsten).

159. **Coprinus plutonius**, Mont., Hist. Nat. Iles Canar. p. 72, pl. 5, Fig. 2.

Pileus *conico-campanulate*, white, costate, *ribs joined by transverse bars*, margin crenate, disc umbonate, even, yellowish, 2–3 cm. across when expanded; gills distant, black; stem 4–6 cm. long, hollow, base incrassated, *white with numerous blackish bands* at regular intervals.

Gregarious on volcanic scoriae. Gomera, Canary Islands.

160. **Coprinus spiralis**, Mont., Hist. Nat. Iles Canar. p. 72, pl. 4, fig. 5.

Pileus *conico-campanulate, acuminate*, margin sulcate for a short distance, ochraceous brown, up to 1 cm. high; gills greyish black;

stem 2–3 cm. high, slender, *spirally twisted and very flexuous, dark coloured.*

On fallen branches in damp places. Canary Islands.

Resembling *Psathyra gyroflexa* in habit. Springing from a dense black mycelium.

161. **Coprinus pilulifer**, Mont., Hist. Nat. Iles Canar. p. 72, pl. 4, Fig. 6.

Pileus *globose, margin minutely toothed*, greyish, pellucid, striate up to the minute, even disc, 4–6 mm. across; gills black; stem 2–3 cm. high, white, naked, very fragile, hollow.

Sandy ground. Canary Islands.

162. **Coprinus phyllophilus**, Karsten, Ryssl. Hattsv. 1, p. 544.

Pileus campanulate, then expanded, glabrous, sulcate, *pale, dingy ochraceous*, becoming sooty, about 1·5 cm. across; gills close to the stem; spores elliptical, 10–13 × 5–7 μ; stem about 6 cm. long, pruinose, then naked, hyaline, pellucid.

Among fallen leaves. Finland.

163. **Coprinus velaris**, Fries, Epicr. p. 253.

Pileus *at first globose*, then hemispherical, coarsely striate, *lurid, disc brownish*, not depressed, up to 2·5 cm. high and wide; gills becoming black, edge white; spores angularly ovate, 7–8 × 5 μ; stem 5–7 cm. long, pellucid, base downy.

On manured ground in gardens, &c. Sweden, Germany, France.

164. **Coprinus pellucidus**, Karsten, Symb. Myc. Fenn. x, p. 61, in Mem. Soc. Fauna et Flora Finl. (1883).

Pileus obovate, then hemispherical, obtuse, sulcate, glabrous, whitish or yellowish, then hyaline and greyish with a darker central spot, 1–2 mm. broad; gills crowded; spores ellipsoid, 7–9 × 4 μ; stem 1·5 cm. long, *flexuous, glabrous*, pellucid.

On cow-dung. Finland.

Closely allied to *C. diaphanus*, Quél.

165. **Coprinus Schroteri**, Karsten, Ryssl. Hattsvampar, 1, p. 345.

Pileus elliptical, then expanded, sulcate, glabrous, *dingy ochraceous, becoming pale, at length sooty-grey*, up to 1 cm. broad; gills brown;

spores angularly subglobose, 13–15 × 8–12 μ; stem 1–2 cm. long, *slightly striate upwards*, minutely pulverulent at first.

On cow-dung. Finland.

Solitary. Allied to *C. Boudieri.*

Var. **proximellus**, Mass. (*Coprinus proximellus*, Karst., Hattsv. 1, p. 544).

Spores elliptical, 10–13 × 5–7 μ; otherwise as in type form.

Manured ground, &c. Finland.

Four species, received during the progress of the work, are in duplicate numbers, signified by an asterisk (*), *thus making a total of* 169 *species.*

Species Imperfectly Described.

Coprinus marculentus, Britz., Hym. Sudb. ix, p. 13.

Coprinus pseudo-nycthemerus, Britz., Hym. Sudb. ix, p. 13.

Coprinus Filholi, Fourc., Rev. Mycol. 1, p. 66.

Excluded Species.

Coprinus pulchrifolius, Peck, 29th Report N. York State Mus. p. 41.

Gills cinnamon, then brown at maturity, and not deliquescent, exclude this species from the genus *Coprinus*, and at the same time suggest the genus *Bolbitius*.

Coprinus Lamottei, Gill., Champ. France, p. 603.

The gills remaining dry, and the brown spores are not in accordance with the conception of the genus *Coprinus*.

Coprinus solifugus, March., in Fries, Hym. Eur. p. 333.

Excluded on account of the yellowish somewhat decurrent gills.

Coprinus setulosus, B. and Br., Journ. Linn. Soc. Vol. xi, p. 561.

The type-specimens are immature, and the gills white, hence there is no evidence that the species is a *Coprinus*. The name should be entirely dropped.

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EXPLANATION OF FIGURES IN PLATES X AND XI.

Illustrating Mr. Massee's paper on Coprinus.

Fig. 1. *Coprinus fimetarius*, var. *macrorhiza*, Fr. Fungus nat. size.

Fig. 2. Basidium with spores of same. ×400.

Fig. 3. *Coprinus gigasporus*, Massee; nat. size.

Fig. 4. Section through pileus of same; nat. size.

Fig. 5. Basidium with spores of same. ×500.

Fig. 6. *Coprinus radians*, Fr., group of plants, showing the radiating mycelium; nat. size.

Fig. 7. Section through pileus of same; nat. size.

Fig. 8. Basidium with spores of same. ×400.

Fig. 9. *Coprinus semistriatus*, Pat., group of plants; nat. size.

Fig. 10. Section of same; nat. size.

Fig. 11. Section through a portion of the hymenium of the same, showing basidia bearing spores, also a cystidium. ×500.

Fig. 12. Spores of same; the spores are lentil-shaped; one of the figures shows the surface of a spore, the other one the edge. ×500 (after Patouillard).

Fig. 13. *Coprinus umbrinus*, Massee; *a*, the plant in a young stage of development, as yet completely enclosed in its white universal veil, which is beginning to crack, owing to the expansion of the pileus; *b*, fully developed plant; the universal veil remains in part as a volva with a free, drooping margin, sheathing the base of the stem, and partly as a central cap, and in the form of torn patches, on the surface of the pileus; nat. size.

Fig. 14. Section through pileus of same; nat. size.
Fig. 15. *Coprinus auricomus*, Pat., cluster of plants; nat. size.
Fig. 16. Section of same; nat. size.
Fig. 17. Portion of hymenium of same, showing a basidium and a cystidium. × 500.
Fig. 18. Spores of same. × 500.
Fig. 19. Portion of surface of pileus of same, showing a projecting filament of Ozonium. × 500 (after Patouillard).
Fig. 20. *Coprinus lagopides*, Karsten; nat. size.
Fig. 21. Section of same species; nat. size.
Fig. 22. Spores of same; highly mag. (after Karsten).
Fig 23. *Coprinus plicatilis*, Fr., plant; nat. size.
Fig. 24. Section of same; nat. size.
Fig. 25. Section through portion of pileus and gills of same, showing the thin flesh of the pileus continuous, at *a*; at *b*, *b'*, the flesh of the pileus is broken, due to the splitting of the gills down the line of the trama, the flesh of the pileus forming scurfy or furfuraceous lines along the crests of the gills at *b*, *b'*. × 100.
Fig. 26. *Coprinus sclerotigenus*, Ellis and Everh., group of Fungi springing from a sclerotium, *a*; nat. size.
Fig. 27. Section of same; *a*, sclerotium; nat. size.
Fig. 28. Spores of same. × 400 (after Ellis).
Fig. 29. *Coprinus tuberosus*, Quélet, springing from a sclerotium, *a*; nat. size.
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Fig. 37. *Coprinus Friesii*, Quélet; nat. size.
Fig. 38. Spores of same. × 400.
Fig. 39. *Coprinus microsporus*, B. & Br.; nat. size.
Fig. 40. *Coprinus rubecula*, B. & Br.; nat. size.
Fig. 41. *Coprinus macropus*, B. & Br.; nat. size.

C. M. del

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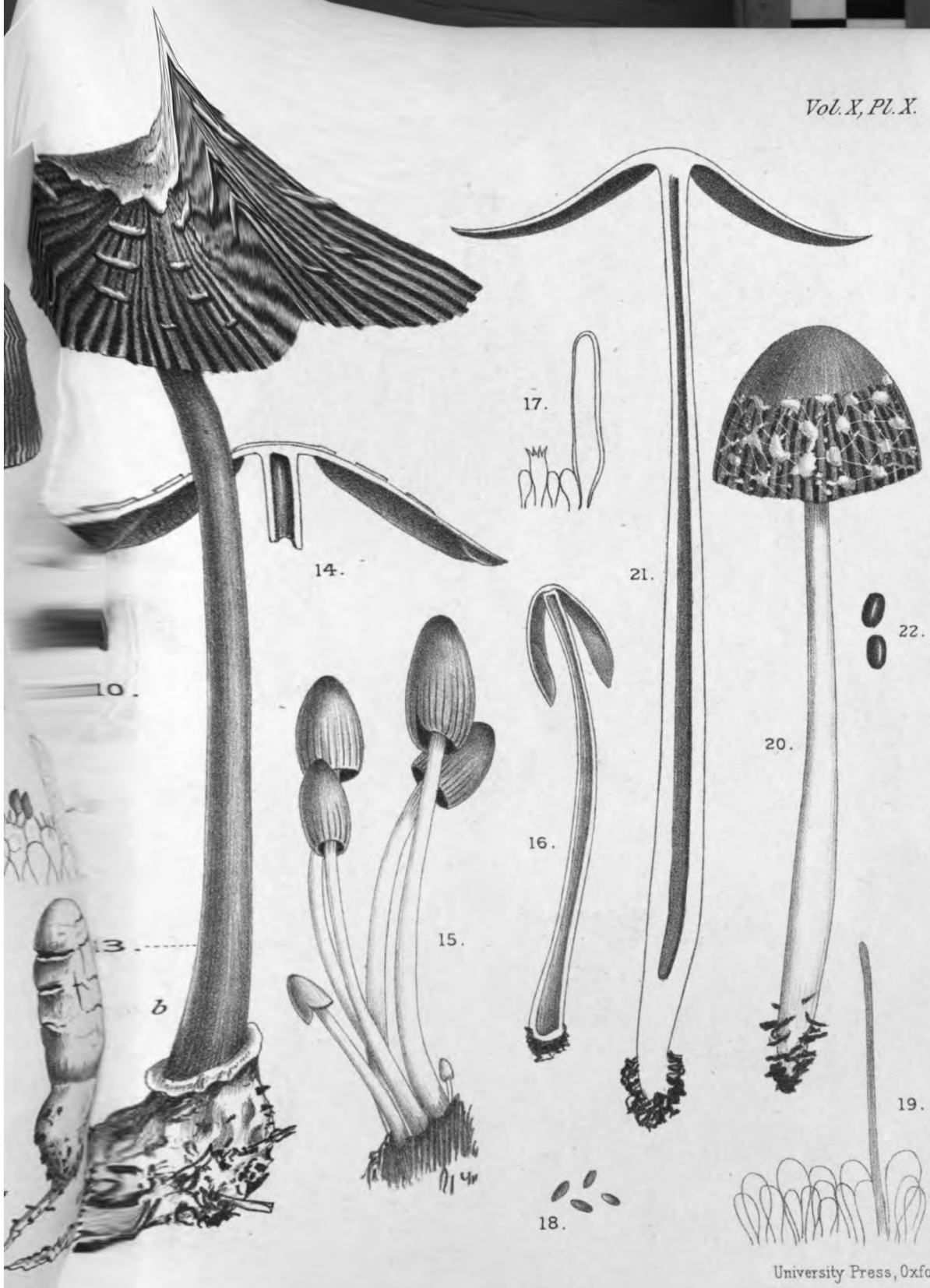

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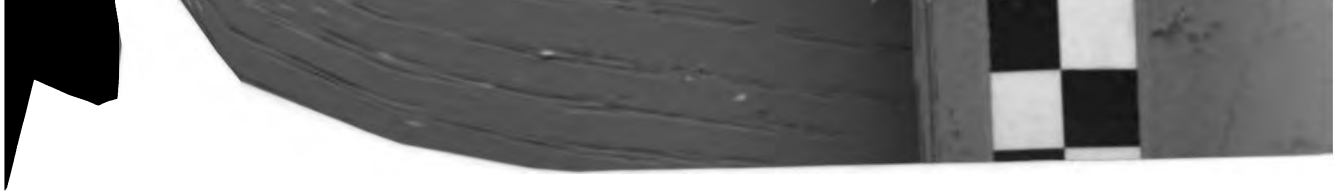

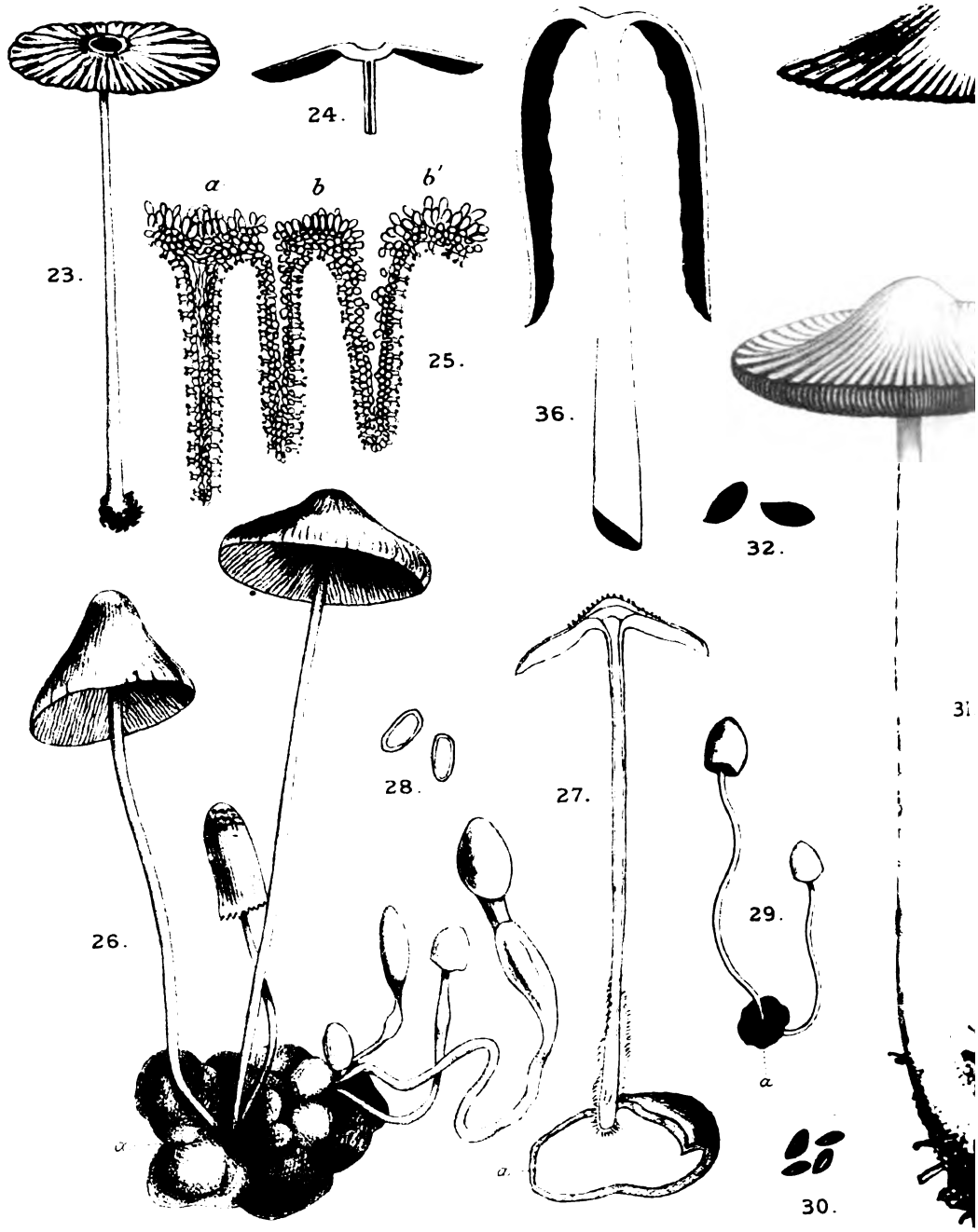

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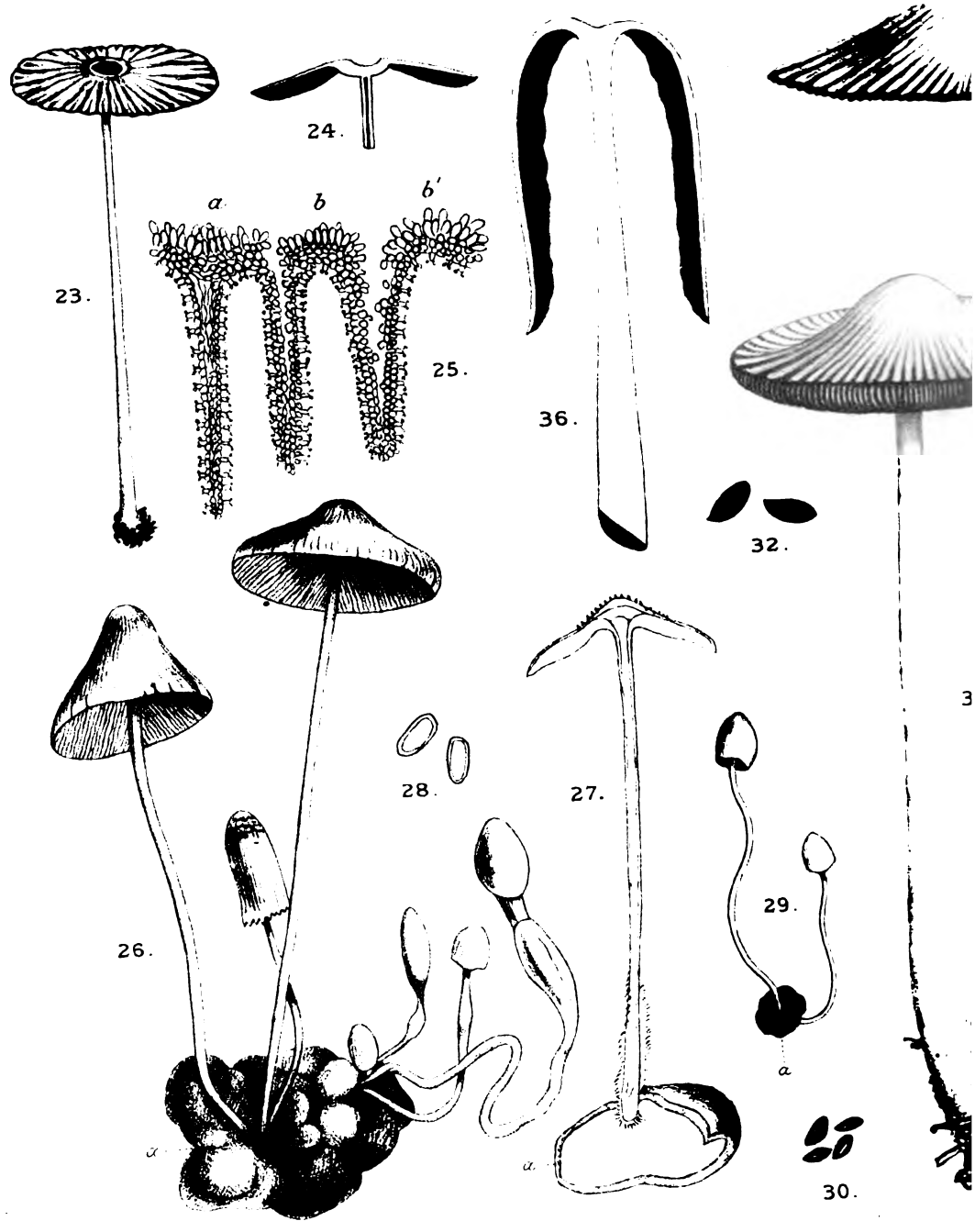

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On the Development of the Cystocarp in Rhodomelaceae (II).

BY

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With Plates XII and XIII.

IN an earlier number of the Annals of Botany (1), I gave an account of some observations on the development of the cystocarp in certain Rhodomelaceae of the genera *Rhodomela* and *Polysiphonia*. The results of these investigations showed that in the four species then under consideration there was a remarkable uniformity in the intimate structure of the procarp at the moment of the fertilization of the trichogyne; but that when spore-formation had set in, there was a varying degree of retrogressive absorption by the sporogenous cell of the cells from which it had been derived. The real nature of the sporogenous cell—whether it constituted in itself the auxiliary cell, as Schmitz averred, or was derived from the auxiliary (pericentral) cell—was left undetermined pending the direct observation of the conjugation of the carpogonium with an auxiliary cell.

The genera *Rhodomela* and *Polysiphonia* represent, however, only two of several groups of genera into which the family of the Rhodomelaceae has been subdivided. Agardh (2) divides

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thirty-nine genera into eleven groups; Schmitz (3), forty-one genera into seven groups. I have therefore been desirous of extending my observations to others of these groups, for the purpose of ascertaining what variations, if any, in the structure of the cystocarp occurred within the limits of this exceptionally large family.

I have not found it possible to successfully make out the structure of the procarp from herbarium-material, and my investigations have therefore been limited to those genera which are represented in the flora of the coasts of North Wales, and of which I could collect material for myself.

The group Laurencieae, Schmitz, is represented in this district by three species of the genus *Laurencia*, Lamx., viz. *L. pinnatifida*, Lamx., *L. hybrida*, Lenor., and *L. obtusa*, Lamx. After some delay I have collected procarp-bearing material of the first-named species. Considering the abundance of the plant along the coast, the female plants seem to be very infrequent. The male plants are more common, but the great majority of the plants consist of individuals bearing tetraspores. Of the other species I have not yet been able to find female plants at all. To the apparent non-occurrence of cystocarp-bearing plants of *L. obtusa*, Harvey (4) has already directed attention. I have examined the procarps of *L. pinnatifida* with the greater interest, because the genus *Laurencia* is altogether excluded from the Rhodomelaceae by Agardh, and retained in the family Chondrieae (5), from which he had, however, already removed the genus *Chondria* itself under the name *Chondriopsis*. Schmitz has divided the family Chondrieae Ag. into two groups of genera, one of which, including the genus *Laurencia*, is transferred to the Rhodomelaceae, the other group constituting the family Bonnemaisoniaceae.

The group Dasyeae is also represented in the district by at least three species of *Dasya*, viz. *D. coccinea*, C. Ag., *D. arbuscula*, C. Ag.; and *D. ocellata*, Harv. *Dasya coccinea* is common, and bears abundant cystocarps. To the examination of this species I have given considerable attention, and I propose to describe the development of its cystocarp in some detail.

Chondria tenuissima, C. Ag., of Schmitz's group Polysiphonieae, is also of common occurrence in the district. In this plant Schmitz had already observed a considerable divergence in the structure of the procarp. In the absence of any figures illustrating his observations, I have thought it desirable to repeat, and if possible extend, the investigation, and particularly to figure the aberrant conditions.

DASYA COCCINEA, C. Ag.

The development of the cystocarp in this plant has already been the subject of a careful investigation by Janczewski (6), and his description is illustrated by no fewer than fifteen figures. As my own observations differ from those of Janczewski in some important particulars, it will be more convenient to deal with these points of divergence together. Schmitz does not appear to have examined *Dasya* at the time of publication of his paper on the fertilization of the Florideae (7), at any rate there is no reference to the genus within the limits of the paper. Later (3 *a*) he seems to have examined *D. elegans*, C. Ag., as that species is given as the type-species of the genus in his systematic review of the Florideae. Later still (8) he made a careful survey of the genus for taxonomic purposes, but this work does not include any description of the structure of the procarp. The genus has not been figured in the publications of Bornet and Thuret.

Dasya differs sharply from most other Rhodomelaceae in the fact that its axis is, as was shown long ago by Kny (9), a sympodium. The allied genus *Heterosiphonia*, Mont., recently re-established by Agardh (10) and Schmitz (8 *a*), alone seems to share with *Dasya* this peculiarity. It might fairly be expected that so profound a difference of structure as that between plants with monopodial and sympodial axes would be accompanied by other differences, possibly in the structure of the procarp.

The procarps of *Dasya coccinea* arise in great numbers close up to the growing apex of the plant. They are usually borne on the fourth joint from the sympodial axis. In these cases

the second joint from the axis gives rise to a branch, which shares in the formation of a secondary sympodial axis of limited growth. Janczewski has observed that occasionally the procarp appears on the second joint itself. The occurrence of a procarp does not, however, prevent the axis branching at that joint (Figs. 1, 2, 3), and the younger branch may even fork once again (Fig. 3). The exhaustion of the axis occasioned by the formation of a procarp seems to set a limit to any considerable development of a sympodium beyond the procarp. It is therefore usual to find only three filaments, each consisting of about a score of cells, which persist until the maturation of the cystocarp. This is the so-called 'involucre' of the species. By some authors the cystocarp is spoken of as 'calcarated.' In other Rhodomelaceae I have invariably found that the procarp is borne upon the second joint of a lateral appendage, now usually called a leaf. The thallus of *Dasya* possesses no such lateral appendages, and the procarps, in common with other parts of the thallus, must be regarded as axial structures. Their occurrence upon a second or fourth joint of a seeming branch has thus no significance in the comparative morphology of the family. Kny found that the branches arise at times upon the third or fifth joint, and it is probable that the procarp will also be found in those situations. The persistence of the filaments in *Dasya* beyond the procarp must, no doubt, be correlated with the fact of its sympodial growth. The leaves of most Rhodomelaceae are fugitive structures, disappearing a short distance behind the growing apex, and when, in these cases, the procarp-bearing leaf withers away beyond the third joint, it may be regarded as exhibiting the same fugitive character.

A common occurrence in *Dasya coccinea* is the relapse of a procarp into an ordinary corticated joint when no spermatium reaches the trichogyne. Only a small proportion of the procarps formed at the apex mature into cystocarps, and every stage of retrogressive transformation may be observed. This is clearly a different process from that which

commonly occurs in *Rhodomela subfusca*, where the unfertilized procarps become transformed into axes of limited growth. It has occurred to me that as branches in various Rhodomelaceae are borne upon the persistent basal cells of the leaves (9), the same process may occur in *Rhodomela*, and the metamorphosis into a branch of a procarp that has remained unfertilized, may be due to the sprouting of the lowest joint.

The procarps of *Dasya* are much more compact and opaque than those of other plants of the family which I have examined. The cell-wall does not swell equally under treatment with glycerine, and I have found the examination of the procarps in an entire condition very tedious and troublesome. Latterly I have adopted the practice of passing material preserved in formaldehyde into a weak solution of gum-arabic, and making sections with the aid of a freezing microtome. For the study of the later stages in the development, when it becomes still more opaque, some method of section-cutting becomes absolutely necessary. Sections cut in frozen gum may afterwards be washed free from the gum, stained in Hoffmann's blue or some other suitable stain, and mounted for examination in strong glycerine. Sections obtained by cutting in paraffin offer no advantages for the purposes here in contemplation, viz. the tracing of the genetic relationship of the contents of the cystocarp. Provided that the sections are not opaque, the thicker they are, the better for the purpose of the investigation. When, however, other problems come to be solved, such as the structure and behaviour of the nucleus in the cells concerned in reproduction, section in paraffin, to which the material readily lends itself, will be found the only available method. The figures of *Dasya*, however, accompanying this paper have been obtained from material fixed in formaldehyde, cut in frozen gum, stained in Hoffmann's blue, and mounted in strong glycerine.

The central cell of the joint which bears the procarps gives off five pericentral cells, and it is the fifth, occupying the median position above (towards the sympodial axis), which gives rise to the essential structures of the procarp. It is

noticeable that whatever be the number of 'siphons' in Rhodomelaceae, whether four as in *Polysiphonia violacea*, or more than twenty as in *Polysiphonia nigrescens*, the number of pericentral cells cut off at the procarpial joint is always five. *Dasya coccinea* has about nine pericentral cells, besides a considerable additional cortex; the central cell cuts off five pericentral cells. The carpogonial branch is four-celled as elsewhere, the terminal cell—the carpogonium—emerging at first as a minute papilla, and afterwards as a greatly elongated trichogyne. The trichogyne is unusually long, because it has to extend far enough to clear the tufts of filaments which terminate the fertile axes. Of the cells of the carpogonial branch, the lowest and the highest (the carpogonium) are in *Dasya* much larger than the two intermediate cells, and none of the four can be so sharply differentiated by staining as is the case in *Rhodomela* and *Polysiphonia* at the corresponding stage.

I have looked carefully into the young procarps for those sterile derivatives of the pericentral cell which are invariably present in the other species, but I am not sure that both of these branches are present, or that even one is always present, at the stage when the carpogonial branch is already fully formed. Occasionally a single inferior cell may be detected (Fig. 1). Both branches may, however, be found a little later: the other pericentral cells have at this stage formed a complete investment for the fifth cell and its derivatives; the procarp never assumes the 'bi-valve' appearance, observable in *Polysiphonia* and elsewhere. There is merely a slight hemispherical swelling on the upper side of the branch, from a pit in which the trichogyne emerges.

Those procarps which advance beyond this stage are relatively few in number, and though the cystocarps appear numerous when mature and visible to the naked eye, the search for them in the intermediate condition just before they become thus visible is wearisome. When the procarp of this stage is examined, it will be found that an internal cavity is forming, owing to the growth of luxuriantly-branched

filaments derived from the pericentral cell. These filaments consist of cells whose walls are thick and transparent, so that the core appears as a mass of dense mucilage in which the tufts of cells are imbedded. At the same time a pair of cells derived from the pericentral cell gives rise to numerous rows which radiate so as to line the entire cavity, converging to the pore. These are the paranematal filaments of other Rhodomelaceae; but they are here formed relatively much earlier in the development of the cystocarp, and play a greater part in the formation of the wall.

When the core of filaments already referred to are examined more closely, they are found to originate, as I have said, in the pericentral cell, and that at two points only. One tuft arises in the inferior position, and branches freely; the other in a lateral position, slightly more luxuriantly branched. Judging from the distance of the cystocarp at this stage behind the growing apex, where the young procarps protrude their trichogynes, an interval of many days (possibly weeks) must have elapsed after the fertilization of the trichogynes before this stage is reached. I was therefore of opinion that these tufts must be sporogenous filaments in a rudimentary condition, particularly as no other structures were apparent which could be regarded as rudiments of carpospores. However, after examining many cystocarps in this condition, two considerations induced me to change this view. First, these filaments arose, as already mentioned, as two branches, one in the inferior position and one in a lateral position. In all other Rhodomelaceae two sterile branches arise in these situations before the fertilization of the trichogyne. In most Rhodomelaceae these consist, one of one cell, another of two cells, and even after fertilization their future development is limited to the production of three new cells, one from each of the three original cells. In *Chondria tenuissima* these branches exist already at the period of fertilization of the trichogyne, as Schmitz has shown, as two tufts of branches of considerable size. The question arose whether the two branches of *Dasya*, which arose almost entirely after fertilization of

the trichogyne, were not the equivalents of these, and not 'carpogenous,' but sterile in their nature. The second circumstance which aroused my suspicion was the fact that in cystocarps at this stage I repeatedly found the carpogonial branch lying intact near the base of these filaments. In other Rhodomelaceae the carpogonium itself, soon after fertilization, becomes disorganized, and cannot be traced; the three remaining cells of the branch atrophy, and are in some cases detached and pushed off on the subsequent formation of spores. Might not the formation of the sterile branches in *Dasya* be delayed, taking place almost wholly after the fertilization of the trichogyne; and might not the fertilized carpogonium remain quiescent pending their formation, and until a suitable stage had been reached for conjugation with an auxiliary cell? Later observations gave a complete answer to these inquiries.

There is no doubt that we have here an analogy with the cases of those Orchidaceae which only form ovules upon the placenta, after pollen has been deposited upon the stigma, the pollen-tube delaying its advent to the ovary until the ovules have been formed. So here, the procarp only proceeds to the further development of the pericarp, and the formation of the 'placental apparatus' including the auxiliary cell, when a spermatium reaches the trichogyne, the activity of the fertilized carpogonium remaining in abeyance until the completion of these operations. Some of the sections illustrating this stage showed that a superior cell was cut off from the pericentral cell, as was found to be the case in most other Rhodomelaceae.

Fig. 4 shows an exceedingly interesting stage in the development of the cystocarp. The two sterile tufts have branched copiously, only a portion of the ultimate filaments being shown in the figure. The paranematal filaments have almost reached the carpostomium. The superior cell has been cut off from the pericentral cell, and at the base of the lateral branch lie the cells of the carpogonial branch. As every pit-connexion in this figure was made out clearly, there is no doubt as to the identity of the cells. A stout process

is, moreover, represented as stretching across between the carpogonium and the superior cell cut off from the pericentral cell. This, I have no doubt, represents the conjugation of the carpogonium and an auxiliary cell, which, though it was inferred that it occurred in Rhodomelaceae, had not previously been observed in any species in the family. The fertilization of an auxiliary cell by the carpogonium has been directly observed in *Dudresnaya*, *Polyides*, *Gloeosiphonia*, and other genera representing different families of the class Cryptoneminae. Schmitz also in his later researches (11) observed this fusion in the Ceramiaceae among the families of the class Rhodomeninae. I have myself during the last summer seen the conjugation-process in *Antithamnion plumula*, Thur.; but I had hitherto failed to observe it in the Rhodomelaceae. The figure is a faithful drawing, by means of a camera lucida, of the appearance presented. A thick short process is seen extending between the carpogonium and the auxiliary cell. In the section it was deeply stained by the Hoffmann's blue, and in this respect, as well as in the general appearance, corresponded closely with the conjugation-process which I had previously seen in *Antithamnion plumula*. The preparation, however, left much to be desired. While the continuity of the process with the auxiliary cell was clear, it was directed obliquely against the carpogonium so as to prevent the continuity with that cell being equally clearly traced. I regret that, in spite of many attempts, I have not been able to repeat this observation in other sections. Once, indeed, in the same situation I found a process, which I have no doubt was the remains of the copulation-tube. In the Ceramiaceae, Schmitz found that a small corner of the carpogonium was cut off, which fused with the auxiliary cell by means of a short tube. Whether anything corresponding to this occurs in *Dasya* could not be determined in the preparation. The observation leaves no doubt, however, that in *Dasya*, at any rate, the cell which plays the rôle of the auxiliary cell, is the one which I had previously called the sporogenous cell. The pericentral cell is not here the auxiliary cell. And I have little doubt now

that this is the rule in the Rhodomelaceae whenever a superior cell is cut off at all. The considerable delay before the conjugation with the auxiliary cell, which takes place in *Dasya*, may indicate some amount of delay in other cases, and my former contention, that the superior cell was not usually cut off until after fertilization of the trichogyne, has less weight as an objection to its being considered an auxiliary in these cases also.

The next figure (Fig. 5) represents the cystocarp one step further in development. The carpogonial branch is still traceable, but now the auxiliary cell has begun to form sporogenous filaments, represented by a dense group of cells seated upon the auxiliary cell. Fig. 6 shows one of these sporogenous filaments (shorn of lateral branches) which has now so developed as to outstrip the tufts of sterile filaments. The cells of these fertile filaments multiply doubtless with great rapidity. The protoplasm is densely granular, the nucleus deeply stained, and the pit-connexions are large and conspicuous. It is characteristic of the cells of these filaments that they present a concave surface upwards, into the well of which the pit-connexion is made. The cells of the sterile filaments are, on the other hand, separated from one another by a dense mucilage, and their pit-connexions are attenuated. No absorption by the auxiliary cell of the pericentral and other cells takes place in *Dasya*, as so often happens elsewhere. The sterile cells may be observed, though in an atrophied condition, even after the carpospores are already recognizable. Though absorption does not take place, there is no doubt that a large number of cells surrounding the central cell are put into requisition to supply the material for the abundant formation of carpospores, which follows upon the fertilization of the auxiliary cell. Among these are the cells of the paranematal filaments. These cells and those below the central cell stain deeply, and seem to be charged with reserve material just before the formation of spores; afterwards they become vacuolated and stain feebly.

The carpospores are ellipsoidal, and are formed in chains

of three or four at the distal ends of the gonimoblast filaments. In other Rhodomelaceae not more than one or two cells usually intervene between the placenta (i.e. the auxiliary cell and its absorbed neighbours) and the carpospore. In some cases the carpospore would seem to be sessile upon the placenta. In *Dasya*, however, long filaments, sometimes pinnately, sometimes subdichotomously branched, interpose eight or ten cells between the placenta and the nearest spore. This stage of the cystocarp is represented in Fig. 7.

This account of the development of the cystocarp in *Dasya coccinea* will be found to differ considerably from that given by Janczewski among a series of observations on the sexual reproduction of Florideae (6). This author found the procarp to contain, as derivatives of the pericentral cell, a 'trichophoric apparatus' of four cells, and two contiguous 'carpogenous' cells. These 'carpogenous' cells are no doubt the rudiments of the sterile branches. In procarps which are left unfertilized Janczewski found, however, that these cells divide to form a 'carpogenic system' of short filaments directed towards the exterior, and constituting the convex portion of the procarp. I am at a loss to account for this statement, as I have found that in unfertilized procarps the earlier convexity is soon lost. Doubtless the cells of the sterile filaments do not die, as, according to Janczewski, do the cells of the 'trichophore.' I have not, however, detected anything in unfertilized procarps corresponding to this description. The writer remarks that the limits of the 'carpogenous system' are not well defined. Further, in fertilized procarps the 'carpogenous' tissue is said to be more vigorous at first, but soon to pass into a condition of rest, during which the vegetative force is exclusively employed in the formation of the pericarp. The pericarp is derived from the two lateral pericentral cells, assisted by neighbouring tissue. This supplementary tissue is, as I have shown, an internal system of paranematal filaments. After the pericarp has attained its definitive aspect, the 'carpogenous system' is described as awaking from its sleep, and producing, at the expense of its terminal cells, the sporogenous filaments.

As I have shown, this is a misunderstanding of the nature of these filaments; the term 'carpogenous system' is a misnomer as applied to them, for they are sterile, and the real sporogenous filaments arise later, not from their terminal cells, but below from the auxiliary cell. The period of rest which Janczewski found to exist may, however, admit of explanation. I have frequently observed cystocarps of the outward shape of mature cystocarps, but with only the tufts of sterile filaments occupying a small space at the base of the cavity. It has occurred to me that these are cystocarps in which the conjugation of the carpogonium with the auxiliary cell has either failed to take place or been greatly delayed. Otherwise the repose is nothing more than the cessation in the growth of the sterile filaments. The function of these sterile branches is partly nutritive perhaps, but they probably also serve the purpose of opening out a cavity for the cystocarp in the dense tissue, by means of their mucilaginous cell-walls. They have, however, with a varying degree of development, a remarkable morphological constancy in all the Rhodomelaceae which I have examined.

Askenasy (12) seems more recently to have described the development of the cystocarp in *Dasya Berkeleyi* (Mont.), J. Ag., and found that the phenomena were similar to what occurred in *Polysiphonia*. *Dasya Berkeleyi* has, however, latterly been removed by Agardh (10 *b*) from *Dasya* to the genus *Heterosiphonia*, Mont. This course is also followed by Schmitz (8 *a*). But while the plant might therefore be expected to depart somewhat from the normal type of *Dasya*, still, inasmuch as it presents the character of a sympodial growth like *Dasya*, it might be expected in the structure of its cystocarp to show affinities to *Dasya* rather than to *Polysiphonia*. Apparently this is not the case. I regret I have not been able to consult Askenasy's paper directly.

In the same work (10) by Agardh as that in the course of which he has re-established the genus *Heterosiphonia*, Mont., he has generally revised the genus *Dasya*, and discussed the structure of the 'nucleus,' in the genus as reconstituted. He refers to

the difference in the appearance of the 'nucleus' when it is less evolved, and consists of shorter, slightly-branched filaments, from that of the more fully-developed 'nucleus' with greatly-prolonged and much-branched filaments. This difference, I do not doubt, is the difference between the cystocarp when it contains only tufts of sterile filaments, and when it contains the larger system of sporiferous filaments. Writing of *Dasya coccinea* itself (10 *a*), he says: 'In Dasya coccinea cuius nucleum fasciculo basali simpliciore constitutum observavi, iam dignoscere licet plures ramos inferne firmiores, quos sensim magis evolutos fieri facilius assumeres.' These 'rami firmiores' are almost certainly the sterile earlier contents of the cystocarp, and, as my figures show, they do not gradually develop into the sporiferous threads.

CHONDRIA TENUISSIMA, C. Ag.

Here, as in *Rhodomela* and *Polysiphonia*, I find the procarp is formed upon one of the so-called leaves, and upon the second joint from the axis. Considering that the axis is relatively stout and strongly corticated, it might seem unlikely that only one stalk-cell intervened between the procarp and the axis. This is, however, the case (Fig. 8), as the cell becomes greatly elongated and attenuated. Janczewski's figures (6 *a*) of this plant err in showing more than one axial cell in the pedicel. Bornet and Thuret's figure (13), however, makes the point clear.

At the time of the fertilization of the trichogyne, the two sterile branches have already attained much the same degree of development as that which they reach long after this stage in *Dasya*. The paranemata have not yet arisen from the central cell, and the four-celled carpogonial branch lies in a lateral position, pushed on one side by the luxuriant growth of the lateral sterile branch. After fertilization the sterile filaments cease to grow almost entirely. The paranemata, however, grow apace, and the upper portion of the cystocarp is formed almost wholly by means of these rows of cells. The

limiting line between the cortex derived from the pericentral cells, and the tissue emerging as paranematal filaments, may be detected at this stage upon the external surface. The carpogonial branch does not immediately atrophy, but remains for some time at the base of the sterile branches. I have failed to observe in this plant the separation of a superior cell from the pericentral cell, or a process of conjugation of the carpogonium with the pericentral cell. A later condition, when an additional (third) branch may be seen to have arisen from the pericentral cell, can be distinguished. This is certainly the gonimoblast-filament arising after conjugation with an auxiliary cell, which in this case may have been the pericentral cell. Schmitz also failed to find in this plant the separation of a distinct auxiliary cell. After the appearance of the gonimoblast-filament there now begins, as Schmitz has pointed out, a process of absorption, starting from the pericentral cell, which attains remarkable dimensions. First, the proximal cells of the sterile branches become continuous with the pericentral cell by the enlargement of the pits and the disappearance of the refractive plates which usually close them. This process does not, however, reach the distal cells at all; and indeed the inferior branch may be seen well defined, though attenuated, at a late stage of spore-formation. In this it resembles the condition of both branches in *Dasya*. The absorption extends from the pericentral cell backwards to the central, and thence to the proximal cells of the paranematal filaments (Fig. 10). By the confluence of these cells there is formed the large, amorphous, multinucleate mass of protoplasm, to which the term 'nucleus' has been more particularly applied by earlier writers. From this mass the pyriform carpospores arise either directly or by the intervention of short gonimoblast-filaments. In no case do carpospores arise from the sterile filaments, or even from their proximal cells which fuse with the 'nucleus,' as long as they are distinguishable from the rest of the protoplasmic mass.

LAURENCIA PINNATIFIDA, Lamx.

The genus *Laurencia* is, as I have stated earlier, placed by Agardh (5) outside the Rhodomelaceae in the family Chondrieae. Schmitz (3) has included it among the Rhodomelaceae, but placed it in a tribe Laurencieae, distinct from the Polysiphonieae, which is made to include *Chondria*. *Chondria tenuissima*, C. Ag., is however *Laurencia tenuissima*, Grev., of the Algae Britannicae (14) and the Phycologia Britannica (4 *a*). I was therefore curious to see what light would be thrown upon the relative position of *Chondria* and *Laurencia* by the comparison of the development of the cystocarp, and I have found that the two plants, *C. tenuissima* and *L. pinnatifida*, exhibit the closest correspondence in the essential elements of the cystocarps. In *Laurencia* the procarps are borne upon the 'leaves' which crowd the depression at the apex of growth; the second joint of these leaves is the one which furnishes the essential procarpial structures; the carpogonial branch is four-celled; two richly-branched tufts of sterile filaments exist already at the moment of fertilization of the trichogyne; as far as I could make out, the gonimoblast-filaments arise here also directly from the pericentral cell, which in that case acts as the auxiliary cell; and the same absorption of neighbouring cells takes place here as in *Chondria*. The paranematal filaments are, however, still more luxuriant in *Laurencia* than in *Chondria*, and line the cavity four or five cells deep. The wall itself also consists of many more layers than is the case in *Chondria*. *Chondria tenuissima* is an annual plant, and has a thin 'cortex' compared with *Laurencia pinnatifida*, which is a perennial, and has a 'cortex' so deep that its 'articulate' character is greatly obscured. Allowing for this difference of habit, the two plants would seem, judging from the structure of the cystocarp, to be very closely allied. It seems to be generally agreed that the cystocarp in Rhodomelaceae usually presents such uniformity that generic characters cannot be founded upon characters derived from it, and while the great similarity of *Laurencia*

and *Chondria* may not now justify their inclusion in one genus, after the plan of earlier authors, it would certainly seem to be a reason for not separating them among different tribes as Schmitz does, much less among different families as Agardh does.

POLYSIPHONIA THUYOIDES, Harv.

This plant belongs to the strongly corticated group of the genus *Polysiphonia*, and is the *Rhytiphlaea thuyoides* of the Phycologia Britannica (4 *b*). I have found the procarps to correspond closely with those of other *Polysiphonia*, excepting that at the period of the fertilization of the trichogyne a superior cell, undoubtedly an auxiliary cell, is already separated from the pericentral cell. I have hitherto assumed that this separation of a superior cell was a consequence of fertilization of the trichogyne. It would, however, appear to be cut off in this case at, or even before, that period; usually it appears immediately after, and in *Dasya* long after, that period.

SUMMARY.

I propose now to briefly summarize the results obtained from the examination of the eight species of Rhodomelaceae, the cystocarps of which I have studied in some detail. The description of the cystocarps of four of these species is contained in a former paper in the Annals (1). The eight species represent five genera, and four of the groups of genera (or tribes) as arranged by Schmitz (3 *b*).

Tribe	Rhodomeleae	*Rhodomela subfusca.*
,,	Laurencieae	*Laurencia pinnatifida.*
,,	Polysiphonieae	*Polysiphonia nigrescens*, *fastigiata*, *violacea*, and *thuyoides*.
		Chondria tenuissima.
,,	Dasyeae	*Dasya coccinea.*

In entering upon a comparison it will be found convenient to take the structures in the following order: the procarp in

general; carpogonial branch; sterile branches; auxiliary cell; paranematal filaments; and the pericarp.

Procarp.—A modified leaf in all cases (except *Dasya*), the second joint of which is the fertile joint. In *Dasya* the procarp is axial.

Carpogonial branch.—Invariably four-celled, arising from the fifth pericentral cell, and curving so as to bring the carpogonium near to the cell from which it arises.

Sterile branches.—Two such branches arising from the pericentral cell—one in the inferior position, one in a lateral position—are invariably present.

In *Rhodomela and Polysiphonia* the inferior branch is one-celled, the lateral branch is two-celled at fertilization of the trichogyne, becoming respectively two-celled and four-celled later.

In *Chondria* and *Laurencia* both branches are luxuriantly branched into tufts of filaments at the time of fertilization, developing but little afterwards, and becoming partially absorbed on spore-formation.

In *Dasya* the two branches are represented but slightly, if at all, at the time of fertilization, but become richly branched afterwards, still before spore-formation sets in.

Auxiliary Cell.—In *Rhodomela, Polysiphonia, and Dasya* there is cut off from the pericentral cell, a superior cell which is the auxiliary cell and conjugates with the carpogonium. In *P. thuyoides* this cell is cut off before fertilization of the trichogyne, usually immediately after, in *Dasya* long after.

In *Chondria* and *Laurencia* it would seem that the pericentral itself acts as the auxiliary cell.

Paranematal filaments.—These derivatives of the central cell are invariably present, and line the cavity of the cystocarp.

In *Rhodomela* and *Polysiphonia* they are few in number and widely separated.

In *Chondria* and *Dasya* they form a continuous layer, often more than one cell deep.

In *Laurencia* they are several cells deep, the inner then becoming more attenuated than the deeper seated.

Fig. 10. A later stage, when the pericentral (auxiliary) cell has absorbed the proximal cells of the sterile branches, and is in process of fusion with the central cell and the paranematal filaments. ×700.

Fig. 11. *a*. Diagrammatic representation of the contents of the procarp in *Rhodomela* and *Polysiphonia* at the time of fertilization of the trichogyne.

b. Diagrammatic sketch of the contents of the procarp of *Chondria* and *Laurencia* at the time of fertilization of the trichogyne.

c. Diagrammatic representation of the contents of the procarp in *Dasya* when spore-formation has begun.

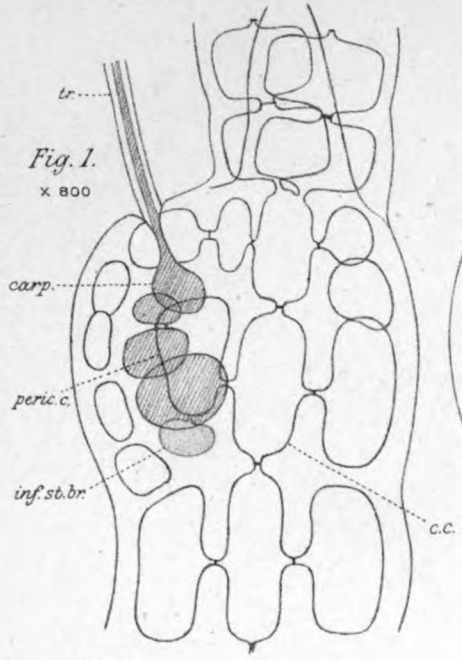

Fig. 1.
× 800

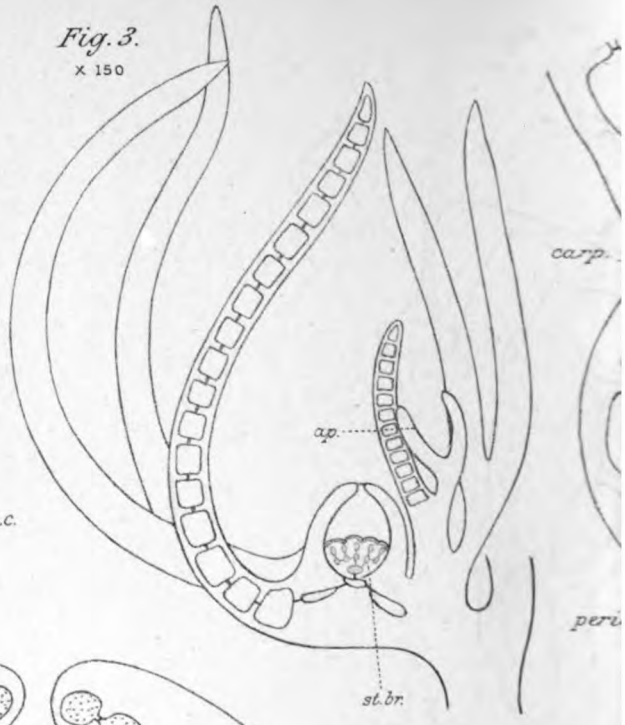

Fig. 3.
× 150

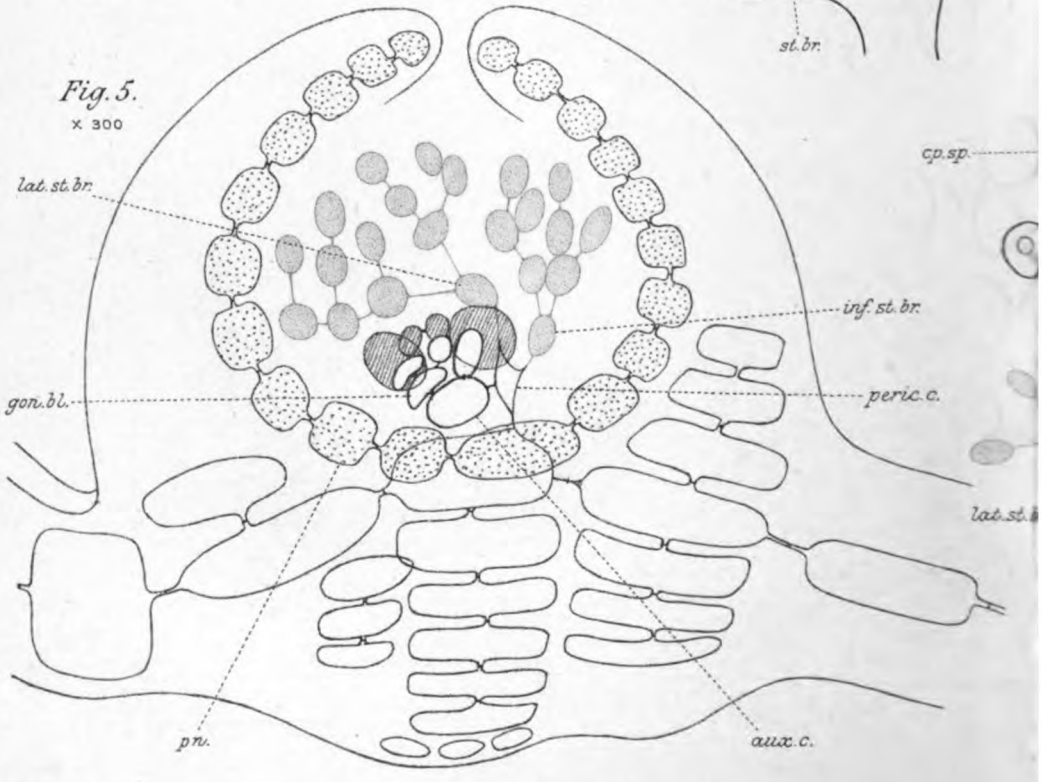

Fig. 5.
× 300

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PHILLIPS.– CYSTOCARP OF RHODOMELACEAE.

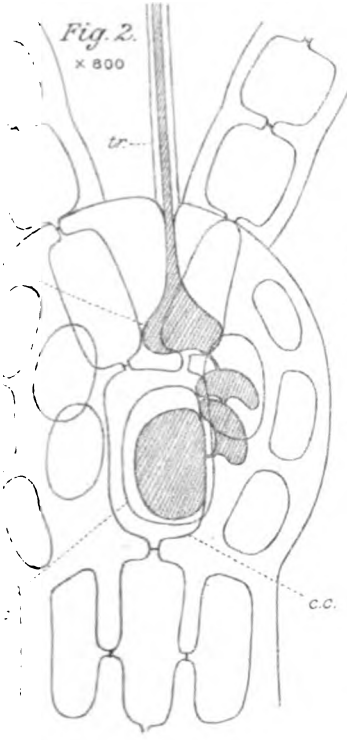

Fig. 2.
× 800

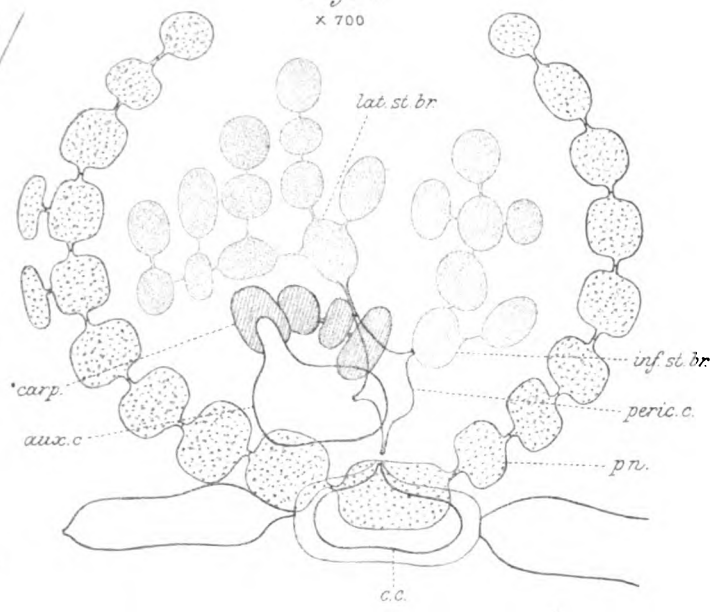

Fig. 4.
× 700

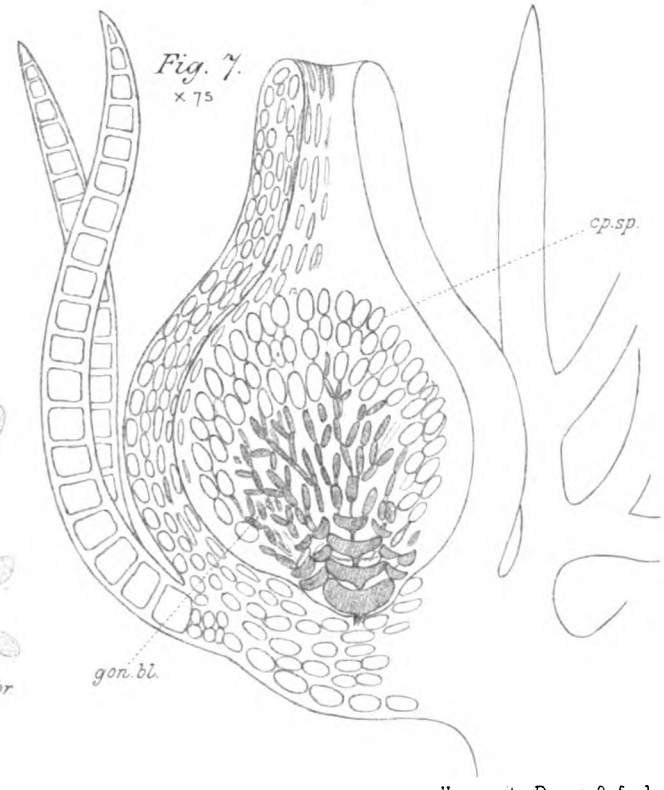

Fig. 7.
× 75

Fig. 6.
× 800

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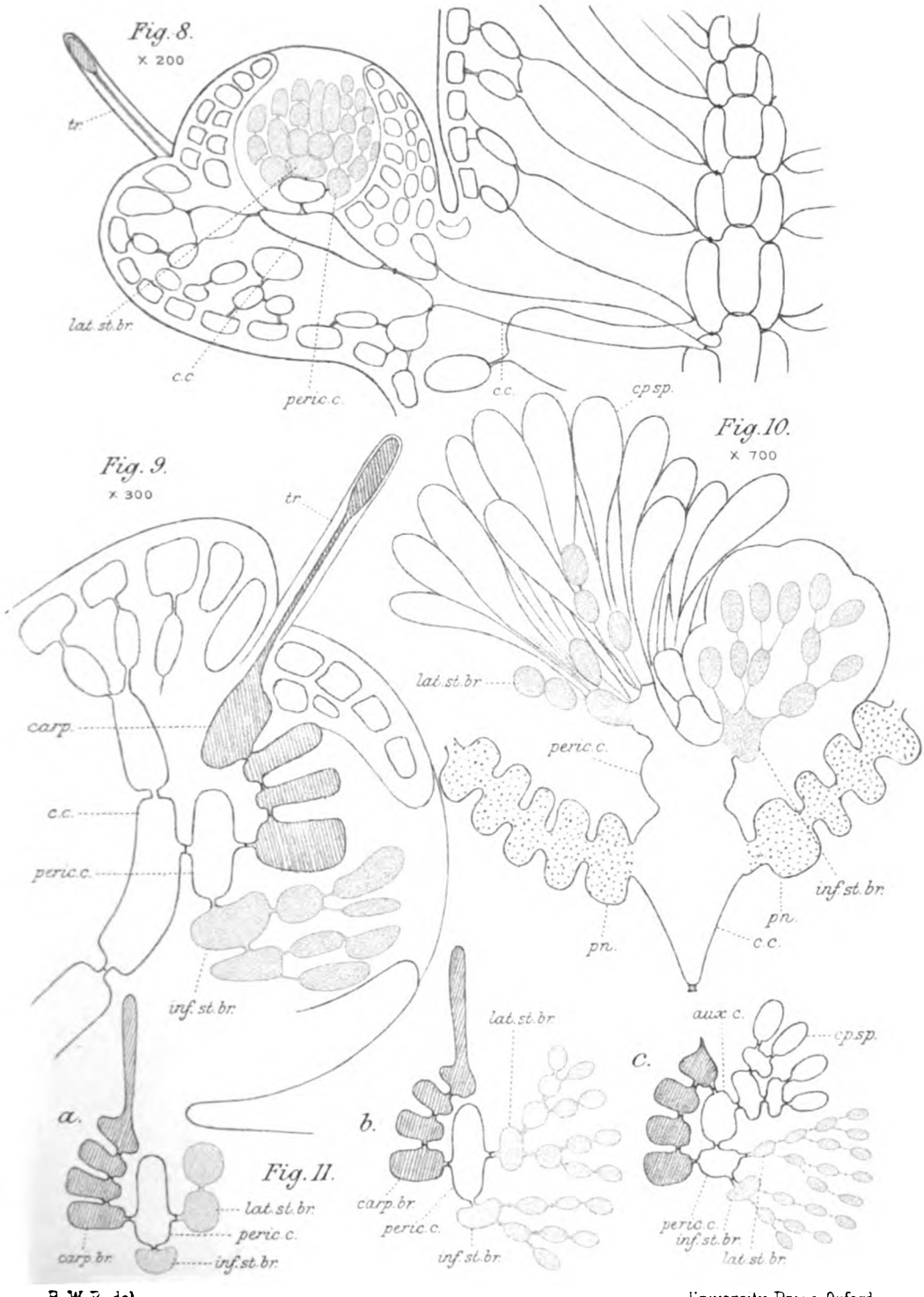
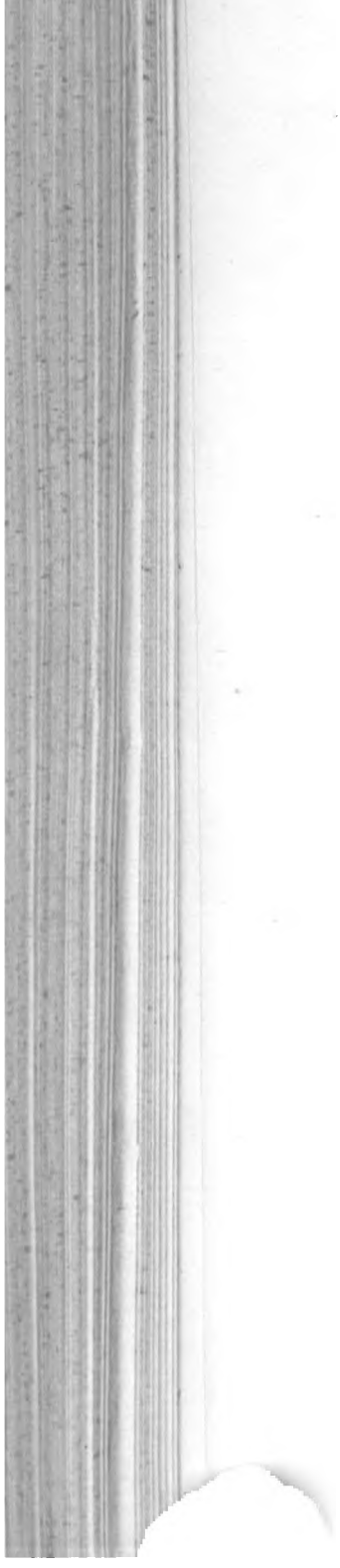


R. W. P. del

University Press, Oxford

PHILLIPS.— CYSTOCARP OF RHODOMELACEAE.



Notes on the Geological History of Monocotyledons.

BY

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With Plate XIV.

THERE are few more interesting problems from a botanist's point of view than the evolution of angiospermous plants. It is not proposed in the present contribution to discuss the lines of development of the Monocotyledons and Dicotyledons, or to take up the question of a separate or common origin of these two groups; but merely to examine the evidence of palaeobotany as to the geological antiquity of Monocotyledons. The records of fossil Angiosperms are in many cases entirely untrustworthy, and stand in need of careful revision. It is often a matter of primary importance to ascertain the relative age, or first appearance in time, of different groups of plants; but unfortunately the statements in palaeobotanical literature are frequently so conflicting and based on such insufficient evidence, that it is by no means easy—in some cases impossible—to arrive at any definite conclusion. It will not, therefore, be by any means a superfluous task to attempt to critically investigate the records of the rocks with reference to the earlier history

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of Monocotyledons, and to endeavour as far as possible to arrive at some conclusion as to the value of the palaeontological evidence.

It is often assumed that monocotyledonous plants are older than Dicotyledons, and this assumption would seem to be supported by the facts of geological history. If, however, we examine more closely into the nature of the palaeontological data, the conclusion is almost forced upon us that no undoubted and satisfactory monocotyledonous plant has so far been recorded from strata older than those in which typical Dicotyledons first occur[1]. To discuss in detail the numerous fossils described as Monocotyledons, would take us far beyond the limits of a single article; it will suffice to refer more especially to some of the better-known earlier records, and to disregard for the present the undoubted representatives of this class discovered in the Upper Cretaceous and Tertiary rocks.

In a paper on Mesozoic Angiosperms, contributed to the Geological Magazine in 1886 by Mr. Starkie Gardner[2], numerous supposed genera of Monocotyledons are fully discussed, and the author is led to the conclusion that these plants may probably be traced back to Triassic, and certainly to the Oolitic rocks. He writes: 'The oldest definite Monocotyledons known are the well-marked Pandanaceous fruits from the Oolites[3],' and quotes as examples the genus *Podocarya* from the Oolite of Charmouth, and *Kaidacarpum* from Inferior Oolite beds at Kingsthorpe, Northampton. The former was originally described and figured by Buckland[4] and referred by him to the Pandanaceae. The specimens are unfortunately not available for re-examination, but an inspection of Buckland's plates in the light of our present

[1] A. C. Seward, Proc. Phil. Soc., Cambridge, Vol. ix, 1896, p. 24.

[2] Geol. Mag. 1886, p. 193.

[3] Ibid. p. 198.

[4] Geology and Mineralogy considered with reference to Natural Theology, London, 1858, p. 466, Pl. 84. Buckland writes that the generic name was suggested by Robert Brown, to whom he owed much of his information on the subject of the fossil.

knowledge of the genus *Bennettites*, leaves little room for doubt that the Charmouth fossil is a well-preserved bennettitean inflorescence. The generic name *Kaidacarpum*, as will be shown later, should probably be replaced by *Araucarites*. In the palaeobotanical volume of Zittel's 'Handbuch der Palaeontologie[1],' Schenk refuses to accept many of the older records, and inclines to the opinion that no trustworthy monocotyledonous plants have been described from Pre-cretaceous rocks. In Lester Ward's 'Sketch of Palaeobotany,' the Monocotyledons are represented as being in existence in Permo-Carboniferous times, at an epoch very much more remote than that in which Dicotyledons first appeared; it is suggested that 'the step from the Monocotyledons to the Dicotyledons is very great, and it seems to have required a vast period of time to accomplish it[2].' Warming, in his admirable Systematic Botany, speaks of the two divisions of the Angiosperms as having probably had a common origin, and adds that—'it is scarcely proved that the Monocotyledons are the older class[3].' It is unnecessary to quote more of the numerous conflicting opinions expressed by botanical writers.

Difficulties and sources of error in the determination of fossil Monocotyledons.

In the Cretaceous and Tertiary strata specimens of silicified Palm-stems are by no means uncommon, and the preservation is such that an accurate diagnosis of the species may frequently be given; with this exception, however, we depend very largely for our knowledge of fossil Monocotyledons on more or less imperfect casts or impressions of structureless stems and leaves. If the tissues of such a plant as *Myeloxylon*, or the petioles of certain Ferns and Cycads, have been only partially preserved, it is conceivable that such structures

[1] Abth. ii. Palaeophytologie. Munich and Leipzig, 1890, p. 357.
[2] Fifth Annual Report, Geol. Surv. U.S.A. p. 448.
[3] Translation by M. C. Potter, London, 1895, p. 273.

might be referred to monocotyledonous species; Schenk[1] points out that the wood of a Conifer, if preserved in patches, as frequently happens as the result of local mineralization, might be erroneously described as monocotyledonous. The close correspondence between the stems of some recent Dicotyledons and those of Monocotyledons, affords sufficient warning as regards the test of histological structure in identifying the stems of angiospermous plants. The parallel venation of monocotyledonous leaves is relied on much too extensively in the determination of fossil specimens. This form of venation is obviously an unsafe guide. Among Dicotyledons, such leaves as those of *Eryngium Lassauxii*, Decne., *E. montana*, Coult., *E. rostratum*, Car., &c., and various dicotyledonous phyllodes and phylloclades might be described as Monocotyledons if found in detached fragments[2]. The linear tapering leaves of these forms of *Eryngium*, with their marginal spines, resemble in a striking degree the leaves of *Pandanus* or certain species of Bromeliaceae. Among the Proteaceae[3], of which the protean nature of the leaves was insisted on by Bentham[4] and again by Bunbury[5] in connexion with sources of error in palaeobotanical determinations, there occur leaf-forms which might well be referred to Monocotyledons. On the other hand, a comparison of the leaves of certain species of *Smilax* with the genera *Pleroma* or *Cinnamomum*, not to mention other examples, shows the danger of following too closely venation-characters. Lindley gave expression to this dicotyledonous form of venation among Monocotyledons by the institution of the family of Dictyogens[6]. The leaves of *Agathis*, certain forms of *Podocarpus* (section *Nageia*), and detached pinnae of Cycadean fronds, may be quoted as possible sources of error where venation is accepted as the most important test.

[1] Die fossilen Pflanzen (Schenk's Handbuch, Vol. iv, 1890), p. 200.

[2] Cf. Drude, in Schenk's Handbuch, Vol. iii (ii), p. 304; see also Henslow in Journ. Linn. Soc. Vol. xxix, 1893, p. 485.

[3] My thanks are due to Mr. Rendle, of the British Museum, for calling my attention to some of the less known forms in this family.

[4] Annual Address, Linn. Soc., 1870, p. 13. [5] Botanical Fragments, p. 310.

[6] The Vegetable Kingdom, 1846, p. 211.

In the pinnae of the Mesozoic Cycad *Ctenis*, which in some species attain a considerable breadth[1], the parallel veins are united here and there by oblique cross-connections, thus closely simulating certain types of monocotyledonous leaves. The superficial resemblance between a palm-leaf, such as *Calamus ciliaris*, Blume, and the frond of a Cycad, would not readily mislead the practised eye of a botanist, but with this form of leaf imperfectly preserved on a piece of sandstone or shale, such a mistake might easily be made. It is not to be wondered at that the older palaeobotanists referred Unger's genus *Cordaites* to the Palms. Schenk[2] has remarked that we do not know at what date *Cordaites* became extinct, and it is quite possible that some of the so-called monocotyledonous leaves should be referred to this genus. Nathorst[3] has demonstrated how the impression of a drifted seaweed on the surface of fine sand may simulate parallel venation. The impression of a radial section of a woody stem of homogeneous structure, such as a Conifer, may be misleading, unless we are able to detect the cross-lines of cells forming the medullary rays. Flattened and imperfect stems of equisetaceous plants, e. g. *Equisetites* and *Schizoneura*, and indeed the leaves of the latter, may well be confounded with angiospermous leaves. Sufficient examples have been cited to illustrate the need of caution, and other instances are supplied by the examples dealt with below.

Palaeozoic and Mesozoic 'Monocotyledons.'

Pothocites. The specimen described many years ago by Paterson[4] under this name, was referred to by Williamson[5]

[1] E. g. those figured by Raciborski in Flora Kopalna-Kracow, 1894, Pls. XVI, XVII, and XVIII.

[2] Die fossilen Pflanzen, p. 200.

[3] Om nagra förmodade Växtfossilier. [Öfversigt Kongl. Vet. Akad. Förhand. 1873, No. 9]. Pls. XV and XVI. See also, Kongl. Svensk. Vetenshaps-Akad. Hand. Vol. xviii, No. 7, 1880, Pls. IX and X.

[4] Trans. Bot. Soc. Edinburgh, Vol. i, 1844, p. 45, Pl. III.

[5] Anomalous Oolitic and Palaeozoic forms of vegetation, 1883, p. 11, Fig. 9.

in 1883, as probably the fructification of an *Asterophyllites* type. More recently Kidston[1] has described several examples of the same fossil, and it is now generally admitted, as this writer shows in his paper, that *Pothocites* is the strobilus of the calamitean genus *Bornia*.

The genus **Palaeoxyris** (*Spirangium*), ranging from the Coal-Measures to the Wealden, and referred by some palaeobotanists to Monocotyledons, is now generally regarded as the egg-capsule of a fish[2]. Other Palaeozoic fossils which have been incorrectly described as Monocotyledons, need not be treated of here as possible pitfalls in phylogenetic investigations.

Passing to the Triassic system, we find such genera as **Yuccites**, **Aethophyllum**, **Echinostachys**, and others recorded as monocotyledonous plants. From the Grès bigarré (Bunter) of the Vosges, Schimper and Mougeot[3] figured and described certain parallel-veined fossils, which in shape and size are spoken of as resembling the leaves of *Yucca*. In *Yuccites vogesiacus*, as represented in Pl. XXX of the memoir on the Vosges plants, we have a very imperfectly preserved impression of torn plant-structures, which apparently possess a parallel venation. This same genus does duty for various specimens described by Zigno[4], and Saporta[5], and others from rocks of Jurassic age. As an example may be mentioned *Yuccites Schimperianus*, which is possibly a portion of a large Cycadean frond[6]. In a recent monograph on the fossil flora of Portugal, Saporta figures a Lower Lias frond as a species of *Yuccites*[7]; but an inspection of the figure will probably suffice to convince botanists of the absence of any real

[1] Ann. Mag. Nat. Hist. Vol. xi [v], 1883, p. 297.

[2] For references to the literature, see Catalogue of the Mesozoic Plants in the British Museum, Wealden Flora, Part II, 1895, p. 224.

[3] Plantes fossiles du Grès Bigarré, 1840.

[4] Flora fossilis formationis Oolithicae, Vol. ii, 1873–85.

[5] Paléontologie Française, Vol. iv, 1891 (Plantes Jurassiques), Pls. VIII–X.

[6] Zigno, loc. cit. p. 7, Pl. XXVI, Figs. 1–4.

[7] Saporta, Flore fossile du Portugal (Direct. trav. géolog. Portugal. Lisbon, 1894), Pl. I, Fig. 24.

justification for the employment of a generic name, and still less of one implying a monocotyledonous affinity.

A fragment figured by the same author in the Flore Jurassique, as an example of *Yuccites*[1], may, as Nathorst has suggested, be a piece of the rachis of a Fern. In no case do we appear to have evidence enough to warrant the use of this generic term for Triassic and Jurassic specimens. The genus *Aethophyllum*[2] stands for certain specimens of which the real nature is still 'very little understood[3].' There seem to be no good reasons for accepting the suggestion that these problematical fossils should be placed among Monocotyledons. The woody stems and linear leaves are not inconsistent with a coniferous plant, but it is useless to speculate as to the affinity of the imperfect and structureless impressions.

Starkie Gardner[4], in the paper previously referred to, has called attention to a fossil described in 1850 by Buckman[5], from the base of the Lias in the neighbourhood of Bristol, as **Najadita**, and expresses the opinion that it should probably be regarded as a Moss resembling the recent *Fontinalis*. He adds in a footnote, that a capsule had been received since the paper was written, but this has not been described or figured. In Pl. V, Fig. 2, accompanying Gardner's paper, a specimen is represented as 'undoubtedly' a monocotyledonous leaf. The long and narrow parallel-veined pinnae of such a Cycad as *Zamia angustifolia*, Jacq., offer a striking resemblance to linear monocotyledonous leaves[6], and it is a bold assumption that the small Purbeck fossil is certainly a Monocotyledon.

In 1851 Bunbury[7] described a specimen from the collection of Mr. Bean under the name of *Calamites Beanii*; the fossil

[1] Paléontologie Française, Vol. iv, Pl. XXII.

[2] Schimper and Mougeot, loc. cit. p. 37.

[3] Solms-Laubach, Fossil Botany, Oxford, 1891, p. 366.

[4] l. c. p. 203.

[5] Quart. Journ. Geol. Soc. Vol. vi, 1850, p. 415.

[6] This specimen, now in the British Museum collection, is represented in Gardner's figure approximately natural size, not half natural size as stated in the plate. It may possibly be equisetaceous, but there is at least no sufficient reason for describing it as a true monocotyledonous leaf.

[7] Quart. Journ. Geol. Soc. Vol. vii, 1851, p. 189.

has since been figured by Gardner[1], who quotes a suggestion by Williamson[2] that it may be a portion of an arborescent monocotyledonous stem. Unfortunately the original specimen has not been found, but the drawing is rather more suggestive of an imperfect cast of an *Equisetites* stem.

The Jurassic fossils figured by Heer[3] as species of **Bambusium**, and the Cretaceous specimens referred by Hosius and von der Marck[4] to a liliaceous genus **Eolirion**, do not call for special discussion; these forms, and the fossil described under the name of **Pitcairnia**, which as Schenk remarks is no doubt a coniferous twig, and other indeterminable examples of fossil plants, cannot be accepted as authentic records of Monocotyledons. There are numerous other instances of fossil stems and leaves described by different writers as Monocotyledons, but to deal with them *seriatim* would be a tedious and unprofitable task. There remain, however, a few examples of fossils recorded as monocotyledonous, which it is important to consider rather more fully.

Aroides. In 1867 Carruthers[5] described a small cylindrical fossil from the Stonesfield slate as *Aroides Stutterdi*, and expressed the opinion that it might reasonably be regarded as part of an aroid spadix similar to that of the recent genus *Xanthosoma*. It has since been suggested that the fossil may possibly be a portion of the anal sac of a Crinoid[6]. Mr. Bather, of the British Museum, who was good enough to examine the specimens, considers that this view cannot be accepted; he is unable to recognize any trace of Echinoderm structure. There are two specimens of this so-called *Aroides* in the British Museum collection[7]; the larger of the two

[1] Geol. Mag. 1886, Pl. IX, Fig. 3. [2] Loc. cit., p. 4.

[3] Flora fossilis Helvetiae, Zürich, 1877, p. 86, Pl. XXX, &c.; also Heer, Contributions à la flore fossile du Portugal [Secc. Trav. Géol. Portugal, 1881], p. 22, Pl. XIX.

[4] Palaeontographica, Vol. xxvi, 1879-80, p. 9, Pl. XXIV, Fig. 6; and p. 93, Pl. XLIV, Figs. 210, 211.

[5] On an Aroideous Fruit from the Stonesfield Slate, Geol. Mag. Vol. iv, 1867, p. 146.

[6] A suggestion quoted by Gardner (loc. cit.), p. 198.

[7] Numbers V. 3442 and 52871, in the Museum Register.

presents an appearance precisely similar to that of the specimen figured by Carruthers, it is sub-cylindrical in form, and in surface view appears to be made up of small plates with irregularly crenulated margins; the smaller and more perfect example referred to the same species, exhibits four rows of hexagonal plates with much more even margins. Without attempting to determine the nature of these fossils, or even to decide whether they are casts of an animal or plant structure, it may be safely asserted that we are not justified in accepting them as proofs of a Jurassic Monocotyledon. Zigno[1] quotes this species as an example of a fossil monocotyledonous plant, but does not offer any comment on the value of the identification.

Kaidacarpum. In a paper on British fossil Pandaneae, written in 1868, Carruthers[2] institutes a new genus, *Kaidacarpum*, and defines it as follows:—'Fruit composed of pyramidal rhomboidal single-seeded drupes, sessile or subsessile on a thickened spadix.' Among other specimens included in this genus, there is the imperfectly preserved fossil represented in Pl. XIV, Fig. 4; this rolled and worn example from the Lower Greensand beds of Potton was named by Carruthers *K. minus*. It must be pointed out that the author of the genus has modified his views as to the nature of some of the species, and inclines to the opinion that they are rather araucarian than monocotyledonous. In Fig. 4 A the external surface shows indistinct traces of spirally arranged depressions; towards the lower end of the cone the stout central axis projects and is marked by more clearly defined and regular pits. Fig. 4 B represents the central axis with portions of the imperfect cone-scales on either side. Had we no better specimens than this to guide us, it would, perhaps, be rash to express a decided opinion as to botanical affinity, but a comparison of the Potton cone with more perfect specimens from the Wealden rocks, brings out very distinctly a close agreement with the female cones of recent

[1] Loc. cit. Vol. ii, p. 2.
[2] Geol. Mag. 1868, Vol. v, p. 153.

Araucarias. The earliest recorded example of one of these supposed pandanaceous fruits is figured in Lindley and Hutton's Fossil Flora [1], and named by them *Strobilites Bucklandi.* A still more perfect example has been figured by Carruthers under the name of *Kaidacarpum ooliticum* [2], from the Great Oolite of Kingsthorpe, Northamptonshire; there is a good specimen of this form in the British Museum collection which shows very clearly the characteristic features [3]. Another example, of what I regard as an imperfectly preserved female cone of *Araucarites*, is represented in Carruthers' well-known memoir on Mesozoic Cycads as probably the male flower of *Bucklandia* [4]. A comparison of the figured specimen with certain cones from the Wealden rocks of Sussex, affords good grounds for regarding it as araucarian. The female cones of recent species of *Araucaria* possess well-marked characters, which enable us to recognize with reasonable probability fossil cones of the same type. In *Araucaria imbricata* the large cones have a short and thick axis, of which the surface is marked with regularly disposed pits or scars of the carpophylls. Each carpophyll is hollow and contains a comparatively large seed, suggesting an angiospermous ovary. In *A. brasiliensis* [5] the stout axis is still more conspicuous, and in *A. Cookii* we have a smaller form conforming to the same type of structure. A section of a large cone of *A. Bidwilli* in the botanical department of the British Museum shows very clearly the nature of the carpophylls and the manner of occurrence of the seeds. If we compare the recent examples with such fossils as *Kaidacarpum ooliticum*, *K. minus*, *Araucarites Huddlestoni* [6], and others, we cannot fail to realize the very striking resemblance. I have elsewhere [7] drawn attention to the similarity of *Kaidacarpum minus* to some Wealden

[1] Vol. ii, Pl. 129.
[2] Loc. cit., Pl. IX, Figs. 1 and 2.
[3] No. 52840 in the Museum Register.
[4] Trans. Linn. Soc. Vol. xxvi, Pl. LIV, Fig. 6.
[5] See Martius' figure in Flora Brasiliensis, Pl. CX.
[6] Carruthers, Quart. Journ. Geol. Soc. Vol. xxxiii, p. 402.
[7] Wealden Flora, Pt. II, p. 190.

cones described by Carruthers as different species of *Cycadeostrobus*[1]. The latter I ventured to speak of under the generic name of *Conites*[2], but a comparison of them with more recently acquired Wealden cones, leads me to refer them all to *Araucarites*. One of the best specimens figured by Carruthers as a cycadean cone is that which he named *Cycadeostrobus Brunonis*[3]; in a transverse section the seeds are clearly seen, and an examination of the specimen tends to confirm my view as to its araucarian affinity. It seems quite impossible to separate under distinct specific types the several pyritized Wealden cones figured in Carruthers' paper on gymnospermous fruits. As a matter of convenience, a specific name must be adopted, but in dealing with imperfect detached cones it is impossible to arrive at any satisfactory conclusions as to different specific forms. Taking Carruthers' species *Cycadeostrobus elegans*, we may briefly define the type as follows:—Cones about 6 cm. in length, and 4 cm. broad; central axis stout, marked with spirally arranged diamond-shaped areas to which are attached broadly triangular carpellary scales, narrowed towards the base, and slightly winged laterally.

This diagnosis is merely intended as a guide to general characteristics, and cannot be accepted as a very precise specific definition. The size of the cones varies considerably, and it is quite possible that more than one species is included in the following list under *A. elegans*. It may tend to remove some of the existing confusion in nomenclature, and to express the conclusions arrived at, if we enumerate those forms which I propose to include in the genus *Araucarites*.

Araucarites elegans (Carr.)[4]. Carruthers, Journ. Bot., Vol. v, 1867, p. 9, Pl. LVII, Fig. 9.

Cycadeostrobus elegans, Carr.

C. truncatus, Carr.[4] Ibid. p. 8, Pl. LVII, Fig. 3.

[1] Carruthers, Journ. Bot. Vol. v, 1867, p. 1. [2] Seward, loc. cit., p. 113.

[3] Carruthers, loc. cit., Pl. LVII, Figs. 4, 5. See remarks on these *Cycadeostrobus* cones in Solms-Laubach's Fossil Botany, p. 92.

[4] The figured specimens are in the British Museum collection.

C. Brunonis, Carr.[1]. Carruthers, Journ. Bot. Vol. v, 1867, p. 10, Pl. LVII, Figs. 4 and 5.

C. ovatus, Carr.[1]. Ibid. p. 8, Pl. LVII, Figs. 1 and 2.

Conites elegans (Carr.). Seward, Wealden Flora, Pt. II, p. 115.

Kaidacarpum minus, Carr. Carruthers, Geol. Mag., Vol. v, 1868, p. 156.

Araucarites, sp. Seward, loc. cit., p. 190.

Bucklandia (male cone)[1]. Carruthers, Trans. Linn. Soc., Vol. xxvi, 1870, Pl. LIV, Fig. 6.

Araucarites ooliticum (Carr.). Carruthers, Geol. Mag., Vol. v, 1868, p. 156, Pl. IX, Figs. 1–6.

Kaidacarpum ooliticum, (Carr.).

Pandanocarpum ooliticum, Zigno. Flor. foss., Oolit., Vol. ii, p. 3.

Araucarites Bucklandi (L. and H.). Lindley and Hutton, Fossil flora, Vol. ii, Pls. I and IX.

Strobilites Bucklandi, L. and H.

Among other fossils described under *Kaidacarpum* may be mentioned *K. suecicum*[2], Nath., *K. sibiricum*[3], Heer, *K. stellatum*[4], Heer, *K. parvulum*[5], Heer, and *K. cretaceum*[6], Heer. In none of these forms have we any satisfactory evidence in favour of a monocotyledonous alliance.

Dracaena Benstedtii. The specimen represented in Pl. XIV, Fig. 3 was originally described and figured by Mackie in 1862[7] as a stem very like that of *Dracaena*. His figure does not convey a very accurate idea of the nature of the fossil.

[1] The figured specimens are in the British Museum collection.

[2] Nathorst, Bidrag till Sveriges fossila flora. II. Floran vid Höganäs, &c. [Kongl. Svensk. Vetenskaps-Akad. Handlingar, Vol. xvi, No. 7, 1878], p. 52, Pl. VI, Fig. 14.

[3] Heer. Flor. foss. Arct. Vol. iv, 1877, p. 84, Pl. XV, Figs. 9–16.

[4] Ibid. p. 85, Pl. XL, Fig. 3 b, and Pl. XV, Figs. 18–20.

[5] Ibid. p. 86, Pl. XV, Fig. 17.

[6] Ibid. Vol. vi, 1882, p. 19, Pl. LXIV, Fig. 9 b.

[7] Geologist, Vol. v, p. 401, Pl. XXII.

This and other specimens from the same locality were named by König *Dracaena Benstedtii*, after Mr. Bensted who discovered the stems in the 'Iguanodon' quarry, but no diagnosis of the species seems to have been published. König's name was adopted by Morris in his Catalogue of British fossils[1]; also by Mantell, who mentions a specimen two and a half feet in length and eight inches in diameter, the surface being marked with 'annular ridges, indicating amplexicaul leaves[2].' In a later work he adds—'until the internal structure of these fossils has been examined, the correctness of this identification is, however, uncertain[3].'

Carruthers in 1868[4] expressed the opinion that Bensted's specimens show a closer resemblance to *Pandanus* than to the stem of *Dracaena*. Gardner[5] alludes to what are probably the same stems as possibly cycadean. I have previously[6] pointed out the close agreement in external form between these Maidstone fossils of Lower Greensand Age and the stems of certain recent Cycads. The example represented in Pl. XIV, Fig. 3, is the same which Mackie described in 1862; the preservation is fairly good; the stem has a girth of 34 cm. and is 9.5 cm. in length along the median line which represents the boundary between two approximately equal branches. The surface is characterized by numerous interrupted transversely running grooves, which curve upwards towards the upper end of the stem where the axis appears to be bifurcating. Numerous small, elliptical, and transversely elongated elevations are scattered over the surface without any regularity of arrangement. Here and there occur patches of a bluish white mineral deposit which do not, however, exhibit any internal structure. In some of the other specimens in the British Museum collection there seem to be traces of a woody structure lining a central cavity occupying

[1] p. 8.
[2] Mantell, Petrifactions and their Teachings, 1851, p. 49.
[3] Ibid., Medals of Creation, Vol. i, 1854, p. 194.
[4] Geol. Mag. 1868, p. 154 (footnote).
[5] Ibid. 1886, p. 201.
[6] Wealden Flora, Pt. II, p. 170.

the axis of the stem; unfortunately the wood-like texture is the result of the crystallization of carbonate of lime, and not organic.

Probably this central cavity represents the pith and a portion of the wood of the original stem.

The largest specimen in the British Museum collection measures 41 cm. in length and 15 cm. in breadth; this and some of the other examples show a number of distinct scars which probably mark the position of lateral buds [1].

In some species of the recent Cycad *Zamia, e.g. Zamia Skinneri*, Warsz., *Z. Loddigesii*, Miq., *Z. Fischeri*, and *Z. pumila*, L. the stem differs very considerably in external characters from the usual cycadean trunk with its characteristic armour of petiole-bases. In this less familiar form the surface is marked by irregular and transverse shallow grooves, and there are frequently found numerous oval corky protuberances scattered irregularly over the surface of the stem [2]. In Pl. XIV, Fig. 2, a portion of a stem of *Zamia Skinneri* is drawn natural size, and in Fig. 1 part of a much branched trunk of *Z. Loddigesii*. A comparison of the two figures with Fig. 3 reveals a fairly striking resemblance. It is not proposed to rely on this correspondence, as regards external features, to the extent of describing the Maidstone fossils as cycadean stems, but simply to draw attention to the possibility of such an identification being correct. In the stem of *Pandanus* there is a distinct similarity to that of *Dracaena Benstedtii*, but on close examination the former shows the leaf-scars and leaf-trace-bundle scars much more distinctly than in the latter; the resemblance of the fossils to stems of *Zamia* is I believe much closer.

The existence of such cycadean stems as those shown in Figs. 1 & 2, seems to have been overlooked by many writers on fossil plants. It need not be pointed out how important it is to pay special attention to the less common and some-

[1] Nos. 8357 and 1765.

[2] The stem of *Cycas siamensis*, Miq., and other Cycads shows in a less degree surface features similar to those of the fossil forms.

what aberrant forms among recent plants, when we are seeking for aids in the determination of fossil specimens.

Instead of retaining the generic name *Dracaena*, I propose to adopt a term which does not imply any particular botanical affinity, and suggest, therefore, that of *Benstedtia*, after the discoverer of the fossil stems. This genus may be defined as follows :—

BENSTEDTIA, gen. nov.

Stems having the surface marked by irregular and interrupted grooves and broader ridges running transversely, with occasional small elliptical protuberances irregularly disposed on the surface of the stem. No distinct leaf-scars; branch-scars may be present, and in addition to smaller lateral branches, a bifurcation of the stem may be indicated by the converging upwards of the transverse lines on the surface of the stem.

Without attempting any specific definitions, we may include under this generic name the stems of the Kentish Rag of Maidstone, and an example recently described from the Wealden rocks of Sussex [1].

CONCLUSION.

In the above incomplete examination of some of the recorded examples of monocotyledonous plants, I have endeavoured to draw attention to the dangerous and misleading practice of assigning generic names, implying definite botanical affinity, to imperfect and in many cases indeterminable fragments. It has been pointed out that the resemblance of the so-called Monocotyledons from Mesozoic rocks to the stems or leaves of recent genera, although in some cases fairly close, is not sufficiently well marked to warrant the conclusion that the fossil specimens should rather be classed with angiospermous than with gymnospermous plants. The discovery of better specimens of certain fossils has supplied us with more complete evidence than was available when these forms were

[1] Wealden Flora, Pt. II, p. 171, Pl. XII, Fig. 5.

first described, and thus it has been found desirable to modify or entirely depart from the determinations of some previous writers. Without venturing to speak dogmatically as to the correctness of some of the suggested alterations, it may be safely urged that it is of extreme importance to critically examine the records of fossil angiospermous species before accepting them as trustworthy contributions towards the history of plant-evolution. The evidence at present available does not, I believe, afford any proof of the existence of Monocotyledons in Pre-cretaceous strata.

EXPLANATION OF FIGURES IN PLATE XIV.

Illustrating Mr. Seward's paper on Fossil Monocotyledons.

Specimens figured Natural Size.

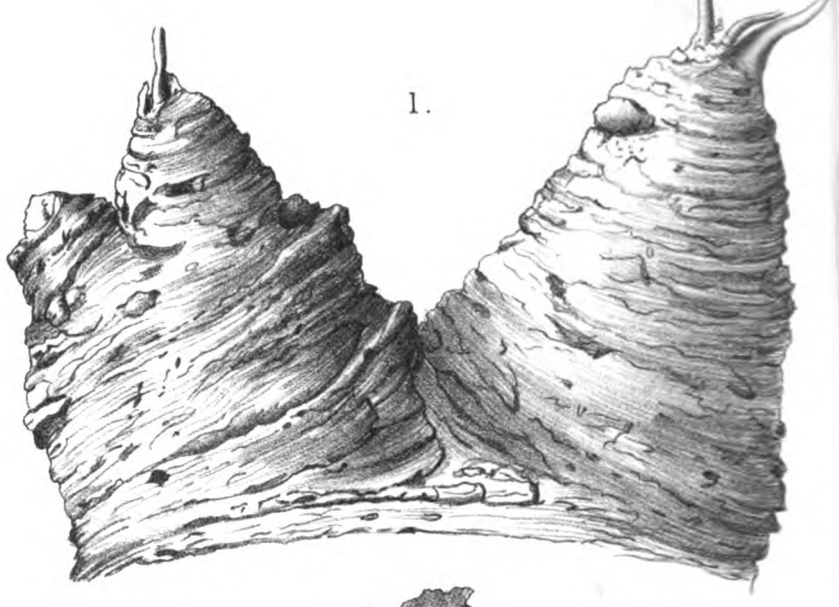
For the drawings reproduced in the Plate, I am indebted to my Wife.

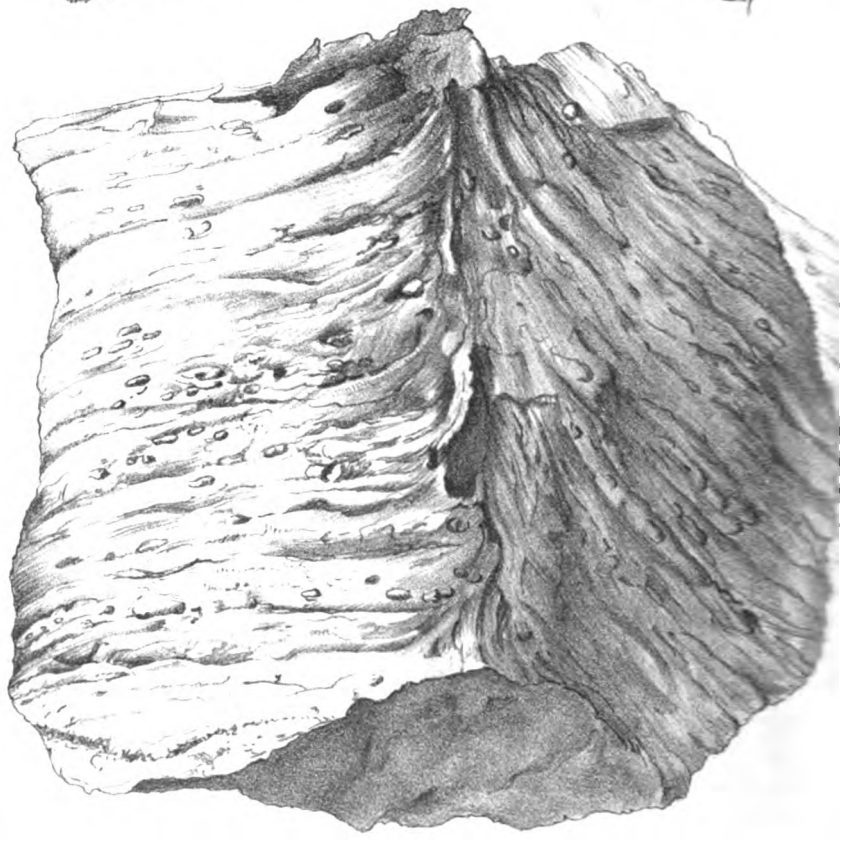
Fig. 1. *Zamia Loddigesii*, Miq. Portion of a much branched stem. (Plant in the Botanic Gardens, Cambridge.)

Fig. 2. *Zamia Skinneri*. Warsz. Portion of erect stem, 26 cm. in girth. (Plant in the Royal Gardens, Kew.)

Fig. 3. *Benstedtia*, sp. Specimen originally figured on a smaller scale by Mackie in the *Geologist*, Vol. v, 1862, Pl. xxii, (No. 1764 in the British Museum Register).

Fig. 4. *Araucarites elegans* (Carr.). Waterworn specimen from the Lower Greensand beds of Potton (Bedfordshire); in the Woodwardian Museum, Cambridge.





M. Seward del.

SEWARD. — FOSSIL MONOCOTYLEDONS.

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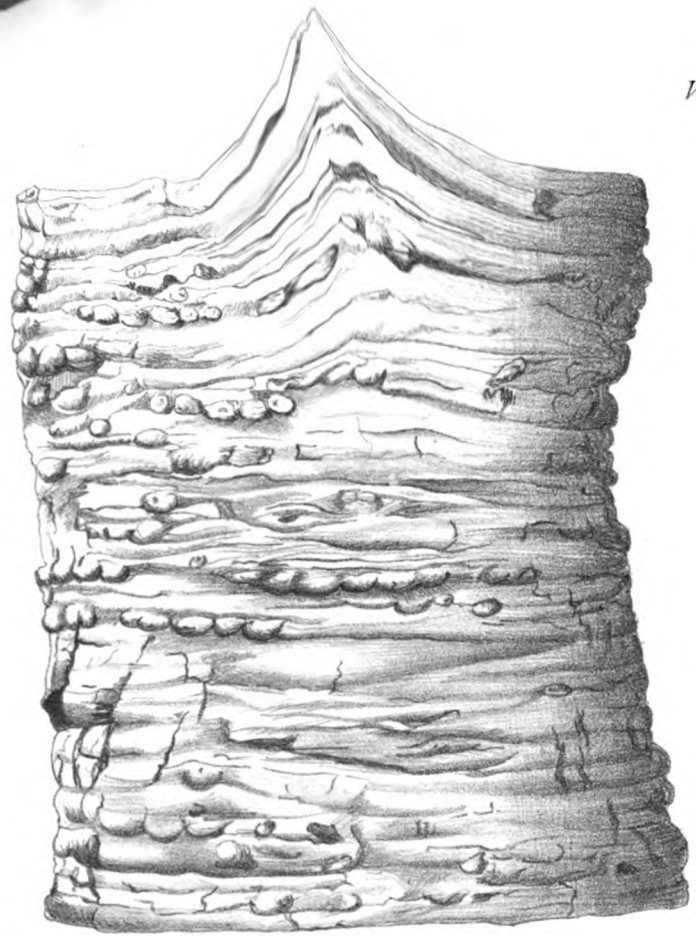

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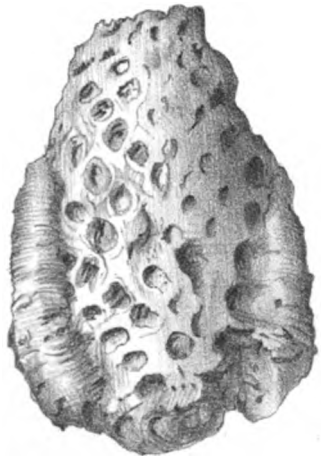

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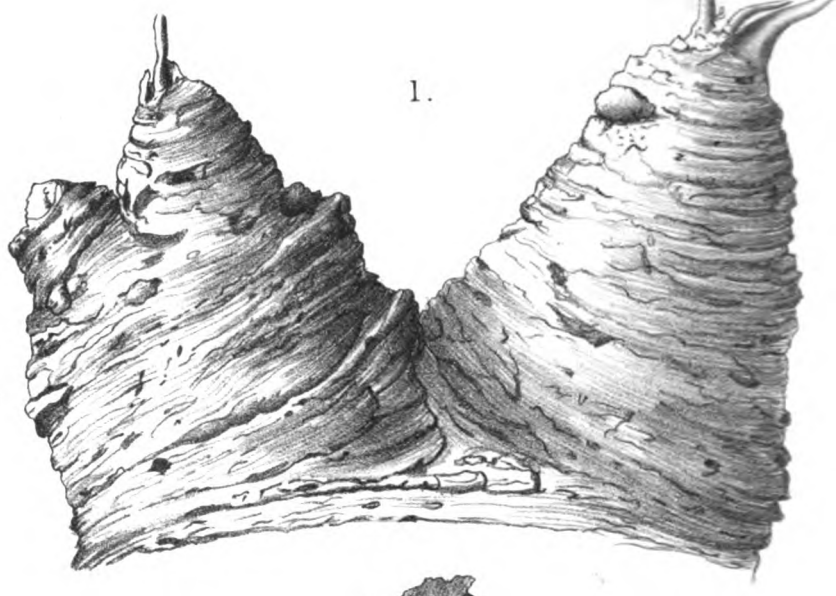

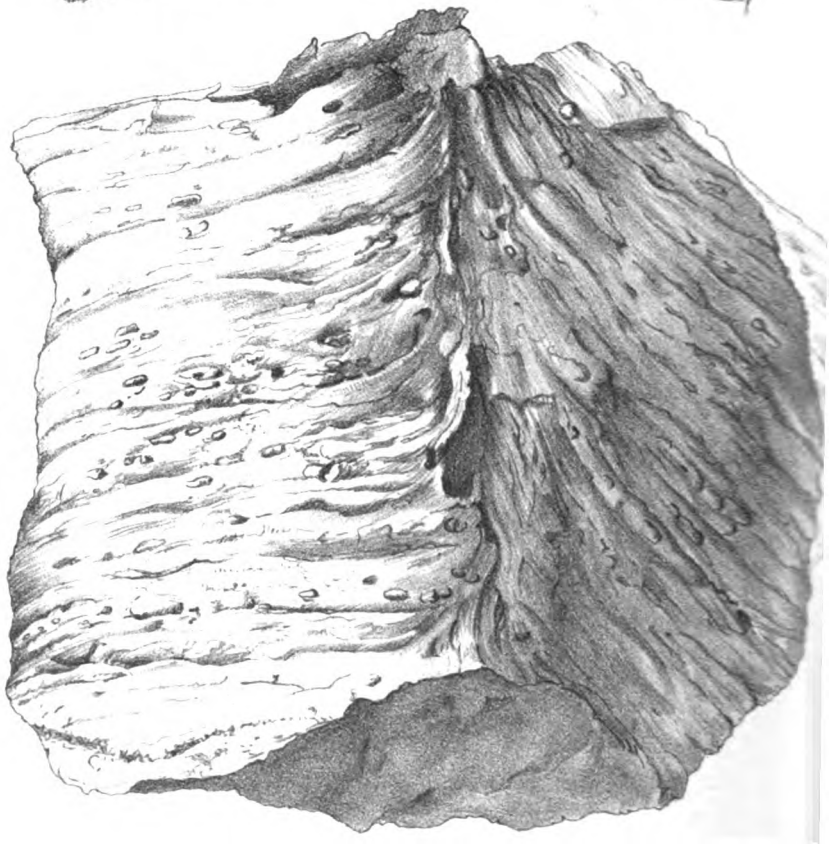

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SEWARD. — FOSSIL MONOCOTYLEDONS.

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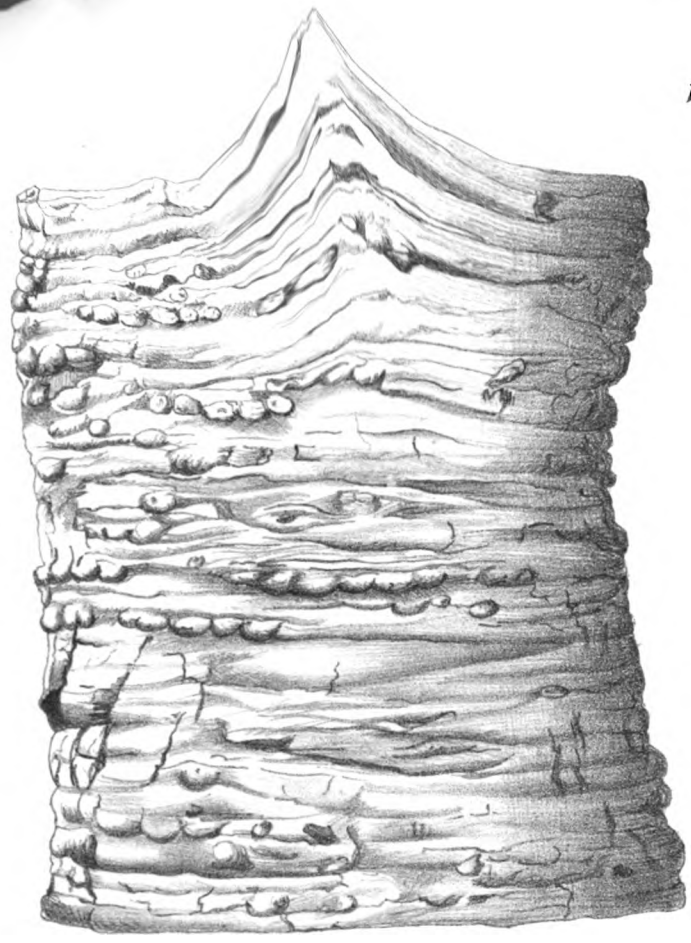

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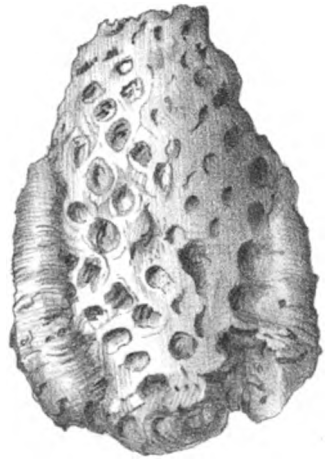

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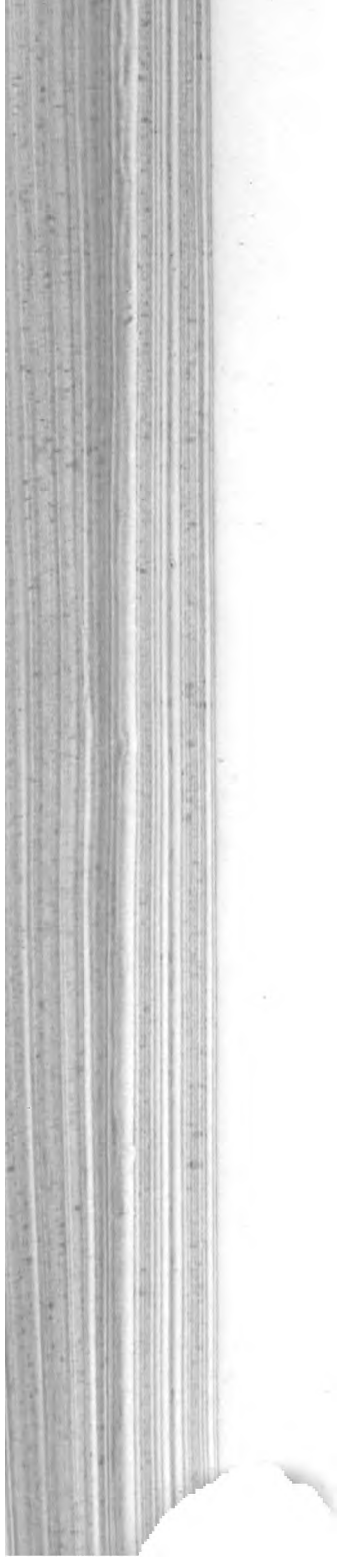

The Flora of Lord Howe Island.

BY

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Principal Assistant in the Herbarium, Royal Gardens, Kew.

INTRODUCTION.

LORD Howe Island is one of the most singular, most beautiful, and most interesting islands in the world; interesting alike on account of its position, its conformation, and its vegetation. It is situated in 31° 30′ S. lat. and 159° E. long., and it is about 300 miles from Port Macquarie, New South Wales. It was discovered by Lieut. Henry Lidgbird Ball, Commander of the *Supply*, in 1788, when on a voyage from Port Jackson to Norfolk Island, for the purpose of founding a convict settlement there, under the governorship of Lieutenant King. It was named after Lord Howe, the First Lord of the Admiralty at the time; and on the return voyage it was surveyed, the results being published in Phillip's and Hunter's accounts of the establishment of the colonies at Port Jackson and Norfolk Island. There are also some references to Howe Island in Surgeon-General White's journal, which contains so much about the natural history of Port Jackson; but, strange to say, the only statement with regard to the vegetation is that the island was very barren.

There is a chart and a view of the island in Governor

[1] The same name was given to a group of islands to the north-east of the Solomon Islands by Captain Hunter, and to one of the islands in the Society group by Captain Wallis.

[Annals of Botany, Vol. X. No. XXXVIII. June, 1896.]

Phillip's book and also a view of the very curious rock, called Ball's Pyramid, which is situated ten miles south-east of the main island. 'They found no fresh water on the island. but it abounds with Cabbage-Palms, Mangrove and Manchineal trees, even up to the summits of the mountains.'

Before proceeding to the history of the botanical exploration of the island. it may be well to give some particulars of its size, conformation, climate, and geology. for which I am mainly indebted to a report by the Hon. J. Bowie Wilson, issued in 1882.

The island is somewhat crescent-shaped, and about seven miles long, with an average width of one mile. On the west side, within the crescent, there is a coral reef, enclosing a lagoon, in which small vessels can safely anchor. There are several adjacent islets, the principal of which are, Mutton Bird Isle, on the east side of the main island; Gower Isle, at the south end; Goat Isle, in the lagoon, and the Admiralty Isles, a small group to the north; not to be confused with the Admiralty Islands in the New Britain archipelago, to the east of New Guinea.

The main island consists of three elevated basaltic masses, connected by low-lying ground of blown coral-sand formation. At the southern end are two circular, steep, flat-topped hills, called Mount Gower and Mount Lidgbird, respectively 2,840 and 2,504 feet high. The central and northern masses of basalt are very much smaller and less elevated, being in the highest parts only 400 to 700 feet high. There is no lack of fresh water in the island; and the soil is sufficiently fertile to support a luxuriant vegetation, even to the summits of the hills. On the low-lying lands the soil is described as exceedingly rich, especially where it has been derived from decomposed basalt, mingled with calcareous sand and decayed vegetable matter. The climate, too, is said to be delicious and equable, with sufficient rainfall; and the vegetation is quite tropical in character.

When first discovered, Lord Howe Island was uninhabited, but thenceforward numerous whaling-ships resorted there for

wood and water; and goats and hogs were soon introduced. In the early days of the Port Jackson settlement, when they were often hard up for food, they used to send to the island for turtles. White relates that the *Supply* on her return from her first voyage to Norfolk Island carried eighteen turtles from the island, the smallest of which did not weigh less than 150 pounds. The Palms, too, were soon much reduced in numbers, being destroyed for the sake of the terminal bud, or 'cabbage,' as it was commonly called, which was the only available vegetable. In 1833 or 1834 half a dozen persons settled on the island, but the occupation was not a permanent one.

The first definite information respecting the flora was obtained by John MacGillivray and William Milne, naturalists to H. M. S. *Herald*, which ship first visited the island in 1853. The former was a zoologist, and the latter a gardener, who was an excellent collector but a very poor scholar, consequently his lengthy manuscript journal in the Kew library contains almost nothing worth transcribing. Both collected and dried plants and transmitted them to Kew; yet they failed to get the Palms and many of the other endemic plants. MacGillivray's list comprises thirty-six species, and Milne's about sixty, including some cellular cryptogams. The vascular plants of these and later collectors are included in Bentham's Flora Australiensis and Mueller's Fragmenta Phytographiae Australis. MacGillivray contributed a short account of their stay in the island to Hooker's Kew Journal of Botany, from which it appears that there were then three families and two or three other persons living in the island. He noted the affinity of the flora with that of Norfolk Island and Australia; also the absence of Coniferae, and the presence of dense forests of Palms. As his observations on the flora are the earliest made by any person possessing the least knowledge of botany, I make the following extract:—

'Here and there is an occasional enormous Banyan-tree, with its singular root-like supporting stems, and some plants of a *Pandanus*, or 'Tent-tree,' as it is here called. My old

friend, *Flagellaria indica*, as usual, is not tied down to the quiet orderly growth of its fellows in the vegetable kingdom, but aspires to paying rambling visits to the summits of the neighbouring trees. What with this, and the Palms, and the Banyans, and the Screw-Pines, and the clumps of parasitical Orchideae and Ferns, the forest scenery struck me as having quite a tropical aspect; and when, after passing some cleared land in a neglected state, overrun with weeds (among which were the ubiquitous *Stellaria media* and *Sonchus oleraceus*), and some patches of rude cultivation, we came in sight of the establishment of one of the settlers. The palm-slab built and palm-leaf thatched cottage and outhouses reminded me of a Malayan or Javanese hamlet. Several species of Ferns occurred here. Besides a *Cyathea*, with a caudex ten or twelve feet in length and six inches in diameter, a very handsome *Hypolepis*, a *Pteris*, a *Litobrochia*, and a widely-spreading *Asplenium*, with fronds six feet in length, were plentiful. A long straggling *Polypodium*, and a *Pleopeltis*, ran over rocks and up the trunks of trees. We saw enormous clumps of a *Platycerium*, high up on the Banyans, and got fine specimens from a tree which had been blown down.'

In June, 1869, Mr. C. Moore, the Director of the Sydney Botanic Garden, spent three days in the island, and collected about 150 species of plants, most of them, unfortunately, without either flower or fruit. A set comprising 100 species was communicated to Kew. Mr. Moore drew up a report on the vegetation, to which was appended a preliminary list of the plants.

In many cases only generic names are given; and in a few instances the specific determinations have since been corrected, either by Moore himself, or by other persons who have studied the plants. I have considered it necessary to cite such erroneous names as synonyms in the following enumeration, because this report, being the best account of the vegetation extant, is likely to be referred to and the names taken up elsewhere. I have been able to verify almost every one of the corrections of the doubtful deter-

minations. This report is so rare that I think I shall be doing a good service by giving almost in full the paragraphs relating to the vegetation.

'Every part of the island is covered with a dense vegetation, the undergrowth being kept comparatively clear by pigs and goats, which are allowed to roam at large. These crop off the lower branches of the trees, and in too many instances, it is feared, have destroyed the smaller kinds of plants altogether. The absence of undergrowth, and the very remarkable scarcity of Ferns and Orchids, in the lowest and richest parts of the island, would indicate a dryness of climate which is not the case, as a drought of any great continuance is seldom or never experienced here, rain being said to be frequent and abundant at all seasons. While the want of undergrowth may be accounted for by the action of pigs and goats, yet the rarity of the classes of plants referred to cannot be so readily explained. Thus, in the rich low flats, extending upwards of three miles, where the trees are thickest and most lofty, only one Orchid—*Dendrobium gracilicaule*, F. Muell.—and five or six Ferns were all that were observed, and these sparingly. At the end of this flat ground towards the east, in gullies near the base of the mountains, and up to their very summit, Ferns increase in numbers, both as regards genera and species. The presence among these of *Trichomanes* and *Hymenophyllum* would dissipate the notion of a very dry atmosphere, and prove at least a greater abundance of moisture in proximity to the mountains than occurs elsewhere. A second and taller species of *Dendrobium* was gathered at a rather high elevation. This, with the former, and a species of *Sarcochilus*, found sparingly upon trees growing on the hilly sides, at the other end of the island, were the only representatives of the family of Orchideae noticed. One of the most remarkable features of the vegetation is the prevalence of Palms, of which there are four species, all of which appear to be as yet undescribed. Two of these, called by the settlers respectively, "Thatch Palm" and "Curly-leaved Palm" [*Howea*], and both sometimes "Cabbage-Palms,"

are very general, and most abundant[1]. They reach to a height of at least 1,000 feet on the side of Mount Lidgbird, at which point their place is supplied by another very distinct species, of a noble appearance, called the "Umbrella Palm" [*Hedyscepe*], from its compact, gracefully-drooping, arched, dome-like, pinnate fronds. The zone of this plant is of limited extent, as it does not reach within some hundreds of feet of the top of the mountain, where a dwarf species [*Clinostigma*], not more than six feet high, also with pinnate fronds, and altogether different from any of the others, occurs in large quantities.

'The *Pandanus*, or "Screw-pine," of which there appear to be two species, marks the vegetation in a peculiar manner wherever it occurs. One species, known to the settlers as the "Tent-tree," *Pandanus Forsteri*, grows plentifully in some parts of the flats, but is more general on the mountain sides, increasing in number as they ascend, and attaining to an elevation of at least 2,000 feet. This often grows to a height of over thirty feet, the lower half of which is usually constituted of spreading stem-like roots, which proceed from the main stem at various heights, and as the earlier roots perish, in a manner almost corresponding with the production of those from above, a clear space is thus left beneath, the plant being supported by these root-props, having a tent-like resemblance. The most remarkable plant, however, upon the island, is a species of *Ficus*, and the only one of the genus found here. Along the whole extent of the flat and richest ground, on the south-west side, this noble tree grows in large numbers—very rarely in exposed situations—but marks distinctly an inner zone of vegetation, being protected on every side by belts of trees of various descriptions. It possesses to an extraordinary degree the branch-rooting characteristics of the famous "Banyan" of India.

'The trees of most frequent occurrence throughout the

[1] These elegant palms are cultivated by thousands in this country now, as they are slow growing and retain their beauty for a long time in pots. In the Palm House at Kew is a handsome fully-developed specimen of *H. Forsteriana*.—W. B. H.

island were *Hibiscus Patersonii*, *Myoporum acuminatum*, called "juniper," and *Ochrosia elliptica*; all most abundant near the coast, and forming for the most part the outer or most exposed belt, the latter being, it is supposed, the "Manchineal" mentioned by Lieutenant Ball, the discoverer of the island. These and a species of *Acronychia*, *Hemicyclia australasica*, the latter remarkable for its bright-coloured foliage and red-coloured fruit, *Olea paniculata*, *Achras costata*, *Pisonia Brunoniana*, *Baloghia lucida*, and a species of *Tetranthera*—constitute at least three-fourths of the tree-vegetation. Climbing-plants were represented by *Marsdenia rostrata*, *Stephania hernandifolia*, *Smilax latifolia*, *Flagellaria indica*, *Ipomoea palmata*, *Ipomoea Pes-caprae*, *Tecoma australis*, and *Canavalia obtusifolia*. Among the more rare and interesting plants, special mention may be made of a magnificent species of *Dracophyllum* discovered by R. D. Fitzgerald, in a valley between the two highest mountains, called Erskine Valley. This magnificient species (perhaps the finest of the genus) being new, will henceforth bear the name of its discoverer, *Dracophyllum Fitzgeraldii*, who describes it as "a tree between forty and fifty feet high, with a trunk at least two feet in diameter. It produced the leaves in tufts at the ends of the branches, and panicles of flowers of a reddish-white colour, from nine inches to a foot long, springing from the centre of the tufts. Altogether it had a strange appearance, growing in a rambling way, the small branches forking like a *Pandanus*, the whole tree having the semblance of producing young Pine-apple plants." A beautiful species of *Randia* (*R. macrophylla*, Moore), with large bright-shining foliage and stipules, was met with in several parts of the island. A singular plant of the Mistletoe kind, *Viscum opuntioides*, found also in Norfolk Island, was observed growing in considerable quantities, but only upon two kinds of trees, *Hemicyclia* and *Elaeodendron*. A most offensive-smelling plant (*Coprosma putida*) was met with both on the high and low grounds. A large Iridaceous plant[1], termed

[1] At the present time (May, 1896) there is a fine plant of this at the south end

the "Wedding Flower," was found sparingly in two or three situations. Of this seed-vessels only were obtained, but the flowers were described as being very beautiful. The leaves were upwards of six feet long, and from two to three inches in breadth. In appearance it resembles a large species of *Moraea*, [*M. Robinsoniana*] but will probably prove to be a new genus.

'At the mouth of a creek or small rivulet, near the base of Mount Gower, *Aegiceras fragrans* was observed for the first and only time, although it is said that this or some other kind of Mangrove grows where another rivulet enters the sea. Along the coast on the northern side *Crinum pedunculatum*, *Juncus maritimus*, *Rhagodia Billardierii*, *Senecio insularis*, *Mesembryanthemum aequilaterale*, *Ipomoea Pes-caprae*, and *Canavalia obtusifolia*, occupied for the most part the sandy ridges raised by the wind from the beach. Curiously enough, on this, the warmest side of the island, the trees and shrubby plants appeared to suffer more from exposure to the sea than they did on the opposite or southern side. There, especially, *Hibiscus Patersonii*, *Ochrosia elliptica*, and *Myoporum acuminatum*, which, as has been before observed, constitute the principal part of the outer belt of tree vegetation, grew to be both good sized and well formed, whilst here they were reduced to a low-sized and nearly impenetrable scrub, the more so as they were usually intermixed with *Guilandina Bonducella*, a sub-climbing prickly shrub. In some parts of the interior, *Verbena bonariensis*, *Ricinus communis*, *Solanum laciniatum*, *Sonchus oleraceus*, and other smaller kinds, evidently foreigners to the soil, had, from neglect, taken almost entire possession of fine tracts of cleared ground, and had become, in other parts, very troublesome weeds.

'Two interesting arborescent Ferns (species of *Alsophila*) were observed in a small valley near the base of Mount Gower, growing in company with *Alsophila excelsa*. In

of the Succulent House at Kew. It is between seven and eight feet high, and has about a dozen vigorous flower-stems.—W. B. H.

the same locality with these, *Trichomanes meifolium*, var. *Bauerianum*, grew plentifully. Another fine species of *Trichomanes*, as well as *Lomaria capensis* and *Hymenophyllum tunbridgense*, were brought from the top of the mountain by one of the settlers, who stated that they all grew there in the greatest profusion, as well as a large and beautiful moss, more than a foot in height [*Spiridens Muelleri*].'

In addition to the collections of Lord Howe Island plants already mentioned, Kew possesses two others, namely, one of sixty species, labelled 'Eclipse Expedition,' communicated by Mr. C. Moore in 1872; and one of ninety-two species, labelled 'Fullagar's Expedition,' purchased through Sir F. von Mueller in 1874. The following year Mueller published a bare list of all the plants known to him from the island, excluding evidently introduced species. This list comprises 182 species of vascular plants.

The Commission, headed by the Hon. J. B. Wilson, which visited the island in 1882, included Mr. J. Duff, of the Sydney Botanic Garden, who furnished a report on the vegetation, from which we learn that the endemic Palms and Tree-ferns were rapidly disappearing, and the latter were in danger of becoming extinct if their removal was not absolutely prohibited. And from some cause not ascertained, many of the fine Banyan trees were dead or dying. It is to be hoped, however, that the recommendations of the Commission that the Colonial Government should not grant further leases, and should take measures to preserve the native vegetation, have been adopted. Among the photographs which embellish Mr. Wilson's report is one of a living Banyan, said to cover an area of three acres! But sad to relate, in many of the other photographs dead trees stand out prominently as white skeletons.

Mr. Duff's report consists mainly of notes on the predominant trees of the island, and such of these notes as are of sufficient interest I have embodied under the respective species in the enumeration.

Some of the party visited the Admiralty Islets, and also

succeeded in landing on Ball's Pyramid, which is about 1,800 feet high, with a base of only three-quarters of a mile in its greatest diameter. Mr. Duff mentions that the plants collected were presented to him, but he gives no names. I have written to Mr. Moore for information on this point, and since writing I have discovered that it has long been his intention to write a Flora of Lord Howe and Norfolk Islands. Of course the present purely geographical paper cannot stand in the way of a descriptive work[1].

ENUMERATION OF ALL THE INDIGENOUS VASCULAR PLANTS KNOWN TO INHABIT THE ISLAND.

I. RANUNCULACEAE.

1. **Clematis glycinoides**, DC. Syst. i, p. 145; Benth. Fl. Austral. i, p. 7; F. Muell. Fragm. ix, p. 76; x, p. 2.

Common in New South Wales and Queensland, and Sir F. von Mueller reports it from New Caledonia.

II. MAGNOLIACEAE.

2. **Drimys Howeana**, F. Muell. Fragm. vii, p. 17.
Drimys insularis, Baill. ex F. Muell. Fragm. ix, p. 76.

Very closely allied to *D. semicarpifolia*, F. Muell., a native of Queensland.

This genus is common to the South American and Australasian regions, and extends to Western Polynesia and the Malay Archipelago, northward to the Philippine Islands.

Moore, in his sketch of the vegetation of the island, cites a second unnamed species, but all the specimens seen belong to one species.

[1] Since writing the above I have heard from Mr. Moore to the effect that he had relinquished the idea; but he hopes yet to be able to send a collector to thoroughly botanize the island, as he believes there is more to be done, and should he succeed he would communicate the materials to me. He failed, however, to answer my question respecting the plants of Ball's Pyramid.

III. MENISPERMACEAE.

3. **Stephania discolor**, Spreng. Syst. iv, Cur. Post. p. 316. *Stephania hernandifolia*, Walp. Rep. i, p. 96; Benth. Fl. Austral. i, p. 57; Maxim. Diag. Pl. Nov. v, t. 2; F. Muell. Fragm. ix, p. 76.

Australia, Polynesia, and tropical Asia and Africa.

IV. CRUCIFERAE.

4. **Lepidium foliosum**, Desv. Journ. Bot. iii (1814), pp. 164, 180; Benth. Fl. Austral. i, p. 86; F. Muell. Fragm. ix, p. 76.

Inserted on the authority of Sir F. von Mueller, though the plant from Lord Howe Island in the Kew Herbarium bearing this name is evidently the same as that referred to *L. ruderale* in the Flora Australiensis. *L. foliosum* is generally spread in extratropical Australia and in Tasmania.

5. **Lepidium ruderale**, L. Sp. Pl. p. 645; Benth. Fl. Austral. i, p. 86; Reichb. Ic. Fl. Germ. ii, t. 10; Engl. Bot. ed. iii, t. 154.

The insular variety is an almost shrubby one, which is widely spread in Australia, and very different in appearance from that common in the northern hemisphere.

V. VIOLACEAE.

6. **Hymenanthera latifolia**, Endl. Prod. Fl. Norf. p. 70; F. Muell. Fragm. ix, p. 77; x, p. 82.

Norfolk Island.

This is probably a variety of *H. Banksii*, R. Br., which would also include the New Zealand *H. crassifolia*, Hook. f., and Sir F. von Mueller has already united them. In this sense the geographical area of the species further includes Tasmania, South Australia, Victoria, and New South Wales.

VI. BIXINEAE.

7. **Xylosma ovatum**, Benth. Fl. Austral. i, p. 108; F. Muell. Fragm. ix, p. 60, et p. 77.

Phoberos? C. Moore, Rep. p. 3.

New South Wales and Queensland.

VII. Pittosporaceae.

8. **Pittosporum erioloma**, C. Moore & F. Muell. in F. Muell. Fragm. vii, p. 139; ix, p. 77.

Endemic.

VIII. Guttiferae.

9. **Calophyllum inophyllum**, L. Sp. Pl. p. 513; Benth. Fl. Austral. i, p. 183; C. Moore in Trans. Roy. Soc. N. S. Wales, v, p. 31; Wight, Ic. t. 77.

Tropical Australia, Polynesia, Malaya, India, and Mascarene Islands.

There is no specimen of this in the Kew Herbarium from the island, although it is included in the manuscript list of the plants presented by Mr. Moore. Nor is it in Sir F. von Mueller's list.

IX. Malvaceae.

10. **Hibiscus diversifolius**, Jacq. Ic. Pl. Rar. t. 551; Coll. ii, p. 307; Benth. Fl. Austral. i, p. 213; C. Moore, Rep. p. 3; F. Muell. Fragm. ix, p. 77; Bot. Reg. t. 381.

Tropics of the Old World.

11. **Hibiscus tiliaceus**, L. Sp. Pl. p. 694; Benth. Fl. Austral. i, p. 218.

Paritium tiliaceum, St. Hil. Fl. Bras. Mer. i, p. 256; F. Muell. Fragm. ix, p. 77; Wight, Ic. Pl. t. 7.

Tropics of the Old World, in islands and maritime districts.

12. **Lagunaria Patersoni**, G. Don, Gen. Syst. i, p. 485; Benth. Fl. Austral. i, p. 218; F. Muell. Fragm. ix, p. 77.

Hibiscus Patersoni, DC. Prod. i, p. 454; Andr. Bot. Rep. t. 286.

Queensland and Norfolk Island.

This is in Mueller's list, but there is no specimen from the island at Kew. Moore states that it was one of the most frequent trees.

Mueller (Fragm. ix, p. 77) includes the genus *Elaeocarpus* (Tiliaceae): '*Elaeocarpus foliatione, quae tantum nota, E. foveolato similis*,' but there is no specimen in any of the collections received at Kew.

X. GERANIACEAE.

13. **Pelargonium australe**, Jacq. Eclog. Pl. t. 100; Sweet, Geran. t. 68; Benth. Fl. Austral. i, p. 298; F. Muell. Fragm. ix, p. 77.

Extratropical Australia, Tasmania, New Zealand, Tristan d'Acunha, and South Africa.

This species, as here limited, presents a wide range of variation.

XI. OXALIDACEAE.

14. **Oxalis corniculata**, L. Sp. Pl. p. 435; Benth. Fl. Austral. i, p. 301; F. Muell. Fragm. ix, p. 77; Reichb. Ic. Fl. Germ. v, t. 199; Wight, Ic. t. 18; Engl. Bot. ed. iii, t. 311.

This is now very widely dispersed, but how far its present area is due to human agency is uncertain.

XII. RUTACEAE.

15. **Melicope contermina**, C. Moore & F. Muell. in F. Muell. Fragm. vii, p. 144; ix, p. 77.

Evodia contermina, C. Moore & F. Muell. loc. cit. vii, p. 144.

Endemic.

Very near the New Zealand *M. ternata*, Forst.

16. **Evodia polybotrya**, C. Moore & F. Muell. in F. Muell. Fragm. vii, p. 143; ix, p. 77.

Endemic.

17. **Zanthoxylum Blackburnia**, Benth. Fl. Austral. i, p. 363; F. Muell. Fragm. vii, p. sine nomine specifico.

Zanthoxylum Howeanum, F. Muell. Fragm. ix, p. 77, sine descriptione.

Blackburnia pinnata, Forst. Prodr. Fl. Ins. Austral. p. 10; Char. Gen. t. 6; Endl. Prodr. Fl. Norf. p. 88.

Norfolk Island and perhaps New Caledonia.

18. **Acronychia Baueri**, Schott, Fragm. Rutac. p. 5, t. 3; Benth. Fl. Austral. i, p. 366; F. Muell. Fragm. ix, p. 77.

New South Wales and Queensland.

XIII. MELIACEAE.

19. **Dysoxylum Fraseranum**, Benth. Fl. Austral. i, p. 381 ; F. Muell. Fragm. ix, p. 77.

Hartighsea Fraserana, A. Juss. in Mem. Mus. Par. xix, p. 262, t. 15.

New South Wales and Queensland.

XIV. CELASTRINEAE.

20. **Elaeodendron australe**, Vent. Jard. Malm. t. 117; Benth. Fl. Austral. i, p. 402 ; F. Muell. Fragm. ix, p. 77.

New South Wales, Queensland, and North Australia.

21. **Elaeodendron melanocarpum**, F. Muell. Fragm. iii, p. 62 ; Benth. Fl. Austral. i, p. 403.

North Australia and Queensland.

XV. SAPINDACEAE.

22. **Cupania anacardioides**, A. Rich. Sert. Astrol. p. 33, t. 13; Benth. Fl. Austral. i, p. 458; F. Muell. Fragm. ix, p. 77.

New South Wales, Queensland, and North Australia.

A plant of this affinity is included in Mueller's list as '*Nephelium a N. semiglauco* [*Cupania semiglauca*] *fructibus majoribus diversum ;*' but there is not a second species from the island in the Kew Herbarium. Tate includes the Australian *N. semiglaucum* in his list without the sign of doubt.

23. **Atalaya coriacea**, Radlk. in Sitzb. Akad. Wiss. Muench. 1878, p. 326 ; F. Muell. Census Austral. Pl. ed. II, p. 41.

Atalaya multiflora, Benth. Fl. Austral. i, p. 463, pro parte.

Endemic.

24. **Dodonaea lanceolata**, F. Muell. Fragm. i, p. 73; ix, p. 77 ; Benth. Fl. Austral. i, p. 475.

Dodonaea viscosa, C. Moore, Rep. p. 3, vix Linn.

South Australia, New South Wales, Queensland, and North Australia.

XVI. LEGUMINOSAE.

25. **Carmichaelia exul**, F. Muell. Fragm. vii, p. 126; ix, p. 77.

Endemic.

With this exception the genus *Carmichaelia* is restricted to the main islands of New Zealand, where the species are numerous and some of them common. *C. exul* is a shrub ten to fifteen feet high, and is singular in the flower-bearing branches being leafy.

26. **Mucuna gigantea**, DC. Prod. ii, p. 405; Benth. Fl. Austral. ii, p. 254; Hook. Bot. Misc. ii, p. 351, Suppl. t. 14; F. Muell. Fragm. ix, p. 77.

Tropical Asia, Australia, and Polynesia.

27. **Canavalia obtusifolia**, DC. Prod. ii, p. 404; Benth. Fl. Austral. ii, p. 256; Benth. in Mart. Fl. Bras. xv, I, t. 48; F. Muell. Fragm. ix, p. 77.

Widely spread in tropical Australia, Polynesia, Asia, Africa, and America, chiefly near the sea.

28. **Vigna lutea**, A. Gr. Bot. Wilk. Exped. i, p. 454; Benth. Fl. Austral. ii, p. 259; F. Muell. Fragm. ix, p. 77; Sinclair, Fl. Hawaii, t. 28.

Tropics of both hemispheres.

29. **Sophora tetraptera**, J. Mill. Ic. Pl. t. 1; Hook. f. Handb. N. Zeal. Fl. p 52; Kirk, For. Fl. N. Zeal. tt. 50–52; F. Muell. Fragm. ix, p. 77; Hemsl. Rep. Voy. *Chall.* Bot. i, pt. 3, p. 32.

Edwardsia chrysophylla, C. Moore, Rep. p. 3.

New Zealand, South America, and Easter Island.

30. **Caesalpinia Bonducella**, Fleming, in As. Res. xi (1810), p. 159; Mart. Fl. Bras. xv, II, t. 21.

Guilandina Bonducella, L. Sp. Pl. p. 545; Benth. Fl. Austral. ii, p. 276; F. Muell. Fragm. ix, p. 77; Lam. Illustr. t. 336.

Very widely spread in maritime districts in the tropics.

XVII. SAXIFRAGACEAE.

31. **Colmeiroa carpodetoides**, F. Muell. Fragm. vii, p. 149; ix, p. 77.

An endemic genus most nearly related to the monotypic New Zealand *Carpodetus*.

XVIII. MYRTACEAE.

32. **Leptospermum flavescens**, Sm. in Trans. Linn. Soc. iii (1797), p. 262; Benth. Fl. Austral. iii, p. 104; F. Muell. Fragm. ix, p. 77; Bot. Mag. t. 2695.

Leptospermum amboinense, Blume, Bijdr. p. 1100; Hook. f. Fl. Brit. Ind. ii, p. 464.

Tasmania, Victoria, New South Wales, and Queensland. Also in Malacca and the Malay Archipelago.

33. **Melaleuca ericifolia**, Sm. in Trans. Linn. Soc. iii (1797), p. 276; Benth. Fl. Austral. iii, p. 159; F. Muell. Fragm. ix, p. 77; Sm. Exot. Bot. t. 34.

Tasmania, Victoria, South Australia, New South Wales, and Queensland.

34. **Acicalyptus Fullagari**, F. Muell. Fragm. viii, p. 15; ix, p. 77.

Eugenia, sp. C. Moore in Trans. Roy. Soc. N. S. Wales, v. p. 31.

Endemic.

Four other species are known; three inhabit Fiji and one New Caledonia.

35. **Metrosideros nervulosa**, C. Moore & F. Muell. Fragm. viii, p. 15; ix, p. 77.

Endemic.

36. **Metrosideros polymorpha**, Gaudich. in Freyc. Voy. Bot. p. 482, t. 85; F. Muell. Fragm. ix, p. 77; Kirk, For. Fl. N. Zeal. t. 119; Hillebr. Fl. Haw. p. 125.

Metrosideros villosa, Sm. in Trans. Linn. Soc. iii (179·), p. 268.

Kermadec Islands, New Caledonia, Tonga, Fiji, Society, Marquesas, Sandwich, and Pitcairn Islands. A characteristic tree almost all over

Polynesia. The genus is represented by about twenty different species in Australia, New Zealand, and Polynesia, two or three in the Malay Archipelago, and one in South Africa.

XIX. PASSIFLORACEAE.

37. **Passiflora Herbertiana** [Ker-Gawl] in Bot. Reg. t. 737; Benth. Fl. Austral. iii, p. 311; F. Muell. Fragm. ix, p. 77.

New South Wales and Queensland.

XX. CUCURBITACEAE.

38. **Sicyos angulatus**, L. Sp. Pl. p. 1013; Benth. Fl. Austral. iii, p. 322; F. Muell. Fragm. ix, p. 77.

A widely-spread weed, common in Australia, New Zealand, and Polynesia.

XXI. FICOIDEAE.

39. **Mesembryanthemum aequilaterale**, Haw. Misc. Nat. p. 77; Benth. Fl. Austral. iii, p. 324; F. Muell. Fragm. ix, p. 77.

All round Australia, and in western South America and California; chiefly on the coast and in salt-marshes. It is closely allied to the South African *M. acinaciforme*, Linn.

40. **Mesembryanthemum australe**, Sol. in Forst. f. Prod. p. 90; Benth. Fl. Austral. iii, p. 324; F. Muell. Fragm. ix, p. 77; Salm-Dyck, Monogr. § 18, fig. 2; F. Muell. Key Vict. Pl. t. 39, figs. *a*, *b*.

Throughout Australia, New Zealand, Chatham and Norfolk Islands; chiefly saline districts. Bentham states (*loc. cit.*) that it is probably not really distinct from the South African *M. crassifolium*, Linn.

41. **Tetragonia expansa**, Murr. in Comm. Götting. vi (1783), p. 13, t. 5; Benth. Fl. Austral. iii, p. 325; F. Muell. Fragm. ix, p. 77; Bot. Mag. t. 2362; Mart. Fl. Bras. xiv, II, t. 71.

Australia except the north, New Zealand, Norfolk Island, Western Polynesia to Japan and South America; chiefly in saline districts.

42. **Tetragonia implexicoma**, Hook. f. Fl. Tasman. i, p. 148; Benth. Fl. Austral. iii, p. 326; F. Muell. Pl. Vict. t. 13;

F. Muell. Key Vict. Pl. t. 40, figs. *a*, *b*; Fragm. ix, p. 77; x, p. 84.

Australia generally, Tasmania, and New Zealand; chiefly in saline districts.

43. **Sesuvium Portulacastrum**, L. Syst. Nat. ed. x, p. 1058; Benth. Fl. Austral. iii, p. 328; F. Muell. Fragm. ix, p. 77; Bot. Mag. t. 1701; Hemsl. Rep. Voy. *Chall.* Bot. i, 3, p. 155; Mart. Fl. Bras. xiv, II, t. 70.

Generally diffused in tropical and subtropical maritime districts.

XXII. UMBELLIFERAE.

44. **Hydrocotyle hirta**, R. Br. in Annal. Sci. Phys. vi (1820), p. 64; Benth. Fl. Austral. iii, p. 339; F. Muell. Fragm. ix, p. 77; Mart. Fl. Bras. xi, I, p. 281, t. 75, f. 2.

Tasmania and Australia, except the north.

This is one of a group of very closely allied species, or forms of one species, scattered over tropical Asia, Africa, and America.

45. **Apium prostratum**, Labill. Relat. i, p. 141; Vent. Jard. Malm. t. 81; Benth. Fl. Austral. iii, p. 372; Labill. Pl. Nov. Holland. i, p. 76, t. 103; F. Muell. Fragm. ix, p. 77.

Australasia, Polynesia, South Africa, and extratropical South America.

XXIII. ARALIACEAE.

46. **Panax cissodendron**, C. Moore & F. Muell. in F. Muell. Fragm. vii, p. 96; viii, p. 280; ix, p. 77.

Endemic.

Allied to the New South Wales *P. Murrayi*, F. Muell. It is locally known as pine wood, on account of its whiteness.

XXIV. RUBIACEAE.

47. **Randia stipulosa**, C. Moore & F. Muell. in F. Muell. Fragm. vii, p. 47; ix. p. 77.

Randia macrophylla, C. Moore, Rep. p. 3, nomen tantum.

Gardenia stipulosa, C. Moore & F. Muell. loc. cit.

Endemic.

Closely allied to *R. Fitzalani*, F. Muell.; a native of Queensland.

48. **Psychotria Carronis**, F. Muell. Fragm. vii, p. 49; ix, p. 77.
Endemic.
Remarkable among Australasian species for the large size of its fruit.

49. **Coprosma Baueri**, Endl. Iconogr. Gen. Pl. xi, t. 111; F. Muell. Fragm. ix, pp. 69 et 77; Kirk, For. Fl. N. Zeal. t. 62.
Coprosma Baueriana, Hook. f. Fl. N. Zeal. i, p. 105; Handb. Fl. N. Zeal. p. 112.
Coprosma lucida, Forst. Char. Gen. p. 138; Endl. Prodr. Fl. Norf. p. 60.
New Zealand and Norfolk Island.

50. **Coprosma lanceolaris**, F. Muell. Fragm. ix, p. 70, et p. 77.
Endemic.
This species is very similar to *C. affinis*, Hook. f. (Fl. Ant. i, p. 21, t. 14), a native of the Auckland Islands.

51. **Coprosma putida**, C. Moore & F. Muell. Fragm. vii, p. 45; ix, p. 77; Moore Rep. p. 2.
Endemic.
This has an exceedingly penetrating odour, in a genus having a very appropriate name. It bears the local name of stinkwood.

XXV. Compositae.

52. **Brachycome segmentosa**, C. Moore & F. Muell. in F. Muell. Fragm. viii, p. 144.
Endemic.
In the place cited, Sir F. von Mueller describes this as distinct from *B. diversifolia*, Fisch. et Mey., and it is probably through a slip of the pen that the latter name appears in his list of Lord Howe Island plants. —Fragm. ix, p. 77.

53. **Olearia Ballii**, F. Muell. Fragm. viii, p. 143.
Aster Ballii, F. Muell. loc. cit. et ix, p. 77.
Endemic.

54. **Olearia Mooneyi**, F. Muell. Fragm. viii, p. 144.
Aster Mooneyi, F. Muell. loc. cit. et ix, p. 77.
Endemic.

This and the preceding are both shrubby, and this attains several feet in height, but there are no arboreous Compositae in the island.

55. **Gnaphalium japonicum**, Thunb. Fl. Jap. p. 311 ; Benth. Fl. Austral. iii, p. 653 ; F. Muell. Fragm. ix, p. 77.

Gnaphalium involucratum, Forst. f. Prod. p. 55 ; Bot. Mag. t. 2582.

New Zealand, Australia, and Eastern Asia.

56. **Gnaphalium luteo-album**, L. Sp. Pl. p. 851 ; Benth. Fl. Austral. iii, p. 653 ; Engl. Bot. ed. iii, t. 742.

Cosmopolitan except the colder regions.

57. **Cassinia tenuifolia**, Benth. Fl. Austral. iii, p. 585 ; F. Muell. Fragm. ix, p. 77.

Endemic.

58. **Wedelia biflora**, DC. in Wight, Contrib. p. 18 ; Benth. Fl. Austral. iii, p. 539 ; F. Muell. Fragm. ix, p. 77.

Wollastonia biflora, DC. Prod. v, p. 546 ; Wight, Ic. t. 1108.

Tropics, except America.

59. **Bidens pilosa**, L. Sp. Pl. p. 832 ; Benth. Fl. Austral. iii, p. 543 ; F. Muell. Fragm. ix, p. 77.

A common weed in almost all warm countries.

60. **Erechthites quadridentata**, DC. Prod. vi, p. 295 ; Benth. Fl. Austral. iii, p. 660 ; F. Muell. Fragm. ix, p. 77.

Senecio quadridentatus, Labill. Pl. Nov. Holland. ii, p. 48, t. 194.

New Zealand, Tasmania, and Australia except the north.

61. **Senecio insularis**, Benth. Fl. Austral. iii, p. 666 ; F. Muell. Fragm. ix, p. 77.

Endemic.

Very distinct, and more nearly allied to the New Zealand *S. glastifolius*, Hook. f., than to any of the Australian species.

XXVI. GOODENIACEAE.

62. **Scaevola Koenigii**, Vahl, Symb. Bot. iii. p. 36 ; Benth. Fl. Austral. iv, p. 86 ; F. Muell. Fragm. ix, p. 77 ; Bot. Mag. t. 2732 ; Hemsl. Rep. Voy. *Chall.* Bot. i, 3, p. 161.

Scaevola Lobelia, Auct. Pl. non Murr.; Hillebr. Fl. Haw. p. 265.

Scaevola latevaga, Hance, in Walp. Ann. ii, p. 1054.

Queensland, North Australia, throughout Polynesia, and in tropical Asia.

XXVII. CAMPANULACEAE.

63. **Lobelia anceps**, L. f. Suppl. p. 395; Benth. Fl. Austral. iv, p. 128; F. Muell. Fragm. ix, p. 77.

Lobelia decumbens, Sims, Bot. Mag. t. 2277.

Australia, except the north, New Zealand, Chatham Island, South Africa, and extratropical South America.

64. **Wahlenbergia gracilis**, Schrad. Blumenb. p. 38, in obs.; A. DC. Monog. Camp. p. 142; Benth. Fl. Austral. iv, p. 137; F. Muell. Fragm. ix, p. 77; Charsl. Wild Fl. Melb. t. 10.

Campanula gracilis, Forst. f. Prod. p. 15; Sm. Exot. Bot. t. 45.

Throughout Australia, New Zealand, Kermadec Islands, New Caledonia, Tonga Islands, Eastern Asia, and South Africa.

XXVIII. EPACRIDEAE.

65. **Leucopogon Richei**, R. Br. Prod. p. 541; Benth. Fl. Austral. iv, p. 186; F. Muell. Veg. Chath. Isd. p. 45; Bot. Mag. t. 3251.

Styphelia Richei, Labill. Nov. Holl. Pl. Sp. i, p. 44, t. 60; F. Muell. Fragm. ix, p. 77.

Australia, except the north, Chatham Island, but not in New Zealand.

66. **Dracophyllum Fitzgeraldi**, F. Muell. Fragm. vii, p. 27, t. 62; ix, p. 77; Wils. Rep. p. 32.

Endemic.

'The largest tree known of the order Epacridaceae, is met with at an elevation of about 1,000 feet, and at this height on the mountains it is a large tree fifty to sixty feet high, with trunks three to nearly five feet in diameter, but it is much smaller as it approaches the top of the mountains, where it is finally reduced to a shrub or small stunted tree of ten to fifteen feet in height.'—DUFF.

XXIX. MYRSINEAE.

67. **Myrsine crassifolia**, R. Br. Prod. p. 534; Benth. Fl. Austral. iv, p. 275; F. Muell. Fragm. viii, p. 48; ix, p. 77.

New South Wales, Queensland, and Norfolk Island.

68. **Myrsine platystigma**, F. Muell. Fragm. viii, p. 48; ix, p. 77.

Endemic.

69. **Aegiceras majus**, Gaertn. Fruct. i, p. 216, t. 46; Benth. Fl. Austral. iv, p. 277; F. Muell. Fragm. ix, p. 77; Wight, Illustr. Ind. Pl. t. 146.

Aegiceras fragrans, Kon.; Moore, Rep. p. 3.

New South Wales, Queensland, North Australia, tropical Asia, New Guinea, Solomon Islands.

XXX. SAPOTACEAE.

70. **Sideroxylon Howeanum**, F. Muell. Cens. Austral. Plants, ed. I, p. 92, 1882.

Achras Howeana, F. Muell. Fragm. ix, pp. 72 et 77.

Achras costata, Moore, Rep. p. 2, non Endl.

Endemic.

This is referred to in Bentham's Flora Australiensis, iv, p. 82, under *Achras australis*, R. Br.

XXXI. STYRACEAE.

71. **Symplocos Stawelli**, F. Muell. Fragm. v, p. 60; ix, p. 77; Benth. Fl. Austral. iv, p. 292 (sub *spicata*).

New South Wales and Queensland.

XXXII. OLEACEAE.

72. **Jasminum didymum**, Forst. f. Prod. p. 3; Benth. Fl. Austral. iv, p. 294; Bot. Mag. t. 6349; F. Muell. Fragm. ix, p. 77.

New Caledonia, Fiji, Tonga and Society Islands, and Northern Australia to Java.

73. **Jasminum simplicifolium**, Forst. f. Prod. p. 3; Benth. Fl. Austral. iv, p. 296; Bot. Mag. t. 980; F. Muell. Fragm. ix, p. 77.

Jasminum gracile, Andr. Bot. Rep. t. 127; Bot. Reg. t. 606; Endl. Prodr. Fl. Norf. p. 55.

Norfolk Island, New Hebrides, Fiji, and Tonga Islands; also the eastern coast of Australia and Norfolk Island.

74. **Notelaea quadristaminea**, Hemsl.

Chionanthus quadristaminea, F. Muell. Fragm. viii, p. 41; ix, p. 77.

Mayepea quadristaminea, F. Muell. Fragm. x, p. 89; Census Austral. Pl. ed. II, p. 156.

Endemic.

75. **Olea paniculata**, R. Br. Prod. p. 523; Benth. Fl. Austral. iv, p. 297; Moore, Rep. p. 3; F. Muell. Fragm. ix, p. 77.

New South Wales, Queensland, and New Caledonia.

XXXIII. Apocynaceae.

76. **Alyxia Lindii**, F. Muell. Fragm. viii, p. 46; ix, p. 77.

Endemic.

77. **Alyxia ruscifolia**, R. Br. Prod. p. 470; Benth. Fl. Austral. iv, p. 308; Bot. Mag. t. 3312: F. Muell. Census Austral. Pl. ed. II, p. 156; Fragm. ix, p. 77.

New South Wales and Queensland, and also Polynesia, according to Mueller.

78. **Alyxia squamulosa**, C. Moore & F. Muell. in F. Muell. Fragm. viii, p. 47; ix, p. 77.

Endemic.

79. **Ochrosia elliptica**, Labill. Sert. Austro-Caled. p. 25, t. 30; Benth. Fl. Austral. iv, p. 310; F. Muell. Census Austral. Pl. ed. II, p. 156; F. Muell. Fragm. ix, p. 77.

Queensland, New Caledonia, Fiji and other islands of the South Pacific. Also in Asia, according to Mueller.

80. **Lyonsia reticulata**, F. Muell. Rep. Burdek. Exped. p. 16; Benth. Fl. Austral. iv, p. 321; F. Muell. Fragm. ix, p. 77.

New South Wales and Queensland.

XXXIV. Asclepiadaceae.

81. **Vincetoxicum carnosum**, Benth. Fl. Austral. iv, p. 331. *Oxystelma carnosum*, R. Br. Prod. Fl. Nov. Holl. p. 462.

New South Wales, Queensland, and North Australia.

82. **Tylophora enervis**, F. Muell. Fragm. ix, pp. 70 et 77.

Endemic.

This is very similar to the Norfolk Island *T. biglandulosa*, F. Muell., syn. *Hybanthera biglandulosa*, Endl.

83. **Marsdenia rostrata**, R. Br. in Mem. Wern. Soc. i (1809), p. 31; Benth. Fl. Austral. iv, p. 339; F. Muell. Fragm. ix, p. 77.

Victoria, New South Wales, and Queensland.

It seems doubtful whether there are two species of this genus in the island, because the flowers of this species, as circumscribed by Bentham, vary considerably in size; and I have seen no authenticated specimen of the following.

84. **Marsdenia tubulosa**, F. Muell. Fragm. ix, pp. 71 et 77.

Endemic.

Mueller (*loc. cit.*) records both this and *M. rostrata* from the island, but all the specimens in the Kew Herbarium are of one species, having, as Bentham states, larger flowers than ordinary *rostrata*, though he refers them to that species.

XXXV. Loganiaceae.

85. **Geniostoma petiolosum**, C. Moore & F. Muell. in F. Muell. Fragm. vii, p. 28; ix, p. 77.

Endemic.

XXXVI. SOLANACEAE.

86. **Solanum aviculare**, Forst. f. Prod. p. 18; Benth. Fl. Austral. iv, p. 447; F. Muell. Fragm. ix, p. 78.

Solanum laciniatum, Ait. Hort. Kew. ed. I, i, p. 247; Bot. Mag. t. 349.

New Zealand, Tasmania, South and East Australia, and Norfolk Island.

87. **Solanum Bauerianum**, Endl. Prod. Fl. Norf. p. 54; F. Muell. Fragm. ix, p. 78.

Norfolk Island.

XXXVII. CONVOLVULACEAE.

88. **Ipomoea biloba**, Forsk. Fl. Aegypt-Arab. p. 44.

Ipomoea Pes-caprae, Roth. Nov. Sp. Pl. p. 109; Benth. Fl. Austral. iv, p. 419; Hemsl. Rep. Voy. *Chall.* Bot. i, 1, p. 51; 2, p. 80; 3, p. 169; F. Muell. Fragm. ix, p. 78.

Ipomoea maritima, R. Br. Prod. p. 486; Bot. Reg. t. 319.

Almost cosmopolitan on sandy seashores in tropical and subtropical countries, including remote islands.

89. **Ipomoea bona-nox**, L. Sp. Pl. ed. II, p. 228; F. Muell. Fragm. ix, pp. 74 et 78.

Now very widely spread in warm countries, but supposed to be indigenous only in tropical America.

90. **Ipomoea grandiflora**, Lam. Tabl. Encyc. i, p. 467; Andr. Bot. Rep. vi, t. 403.

Ipomoea longiflora, R. Br. Prod. p. 484; Benth. Fl. Austral. iv, p. 418.

Queensland, North Australia, Polynesia, tropical Asia and Africa.

This is not in Mueller's list, but there is a specimen in the Kew Herbarium received from him.

91. **Ipomoea palmata**, Forsk. Fl. Aegypt-Arab. p. 43; Benth. Fl. Austral. iv, p. 415; F. Muell. Fragm. ix, p. 78.

Ipomoea pendula, R. Br. Prod. p. 486; Andr. Bot. Rep. t. 613; Bot. Reg. t. 632.

Tropics of both hemispheres.

92. **Calystegia marginata**, R. Br. Prod. p. 484; Hook. f. Fl. N. Zeal. t. 48.

Convolvulus marginatus, Spreng. Syst. i, p. 603; Benth. Fl. Austral. iv, p. 430; F. Muell. Fragm. ix, p. 78; x, p. 113.

Calystegia affinis, Endl. Prodr. Fl. Ins. Norf. p. 51, fide F. Muell. loc. cit.

New Zealand, South-eastern Australia, and Norfolk Island.

93. **Calystegia Soldanella**, R. Br. Prod. p. 483.

Convolvulus Soldanella, L. Sp. Pl. p. 159; Benth. Fl. Austral. iv, p. 431; F. Muell. Fragm. x, p. 113; Engl. Bot. ed. III, t. 925.

Convolvulus sepium, L. var. *Soldanella*, F. Muell. Fragm. ix, p. 78.

Cosmopolitan on seashores in temperate and subtropical countries.

XXXVIII. GESNERACEAE.

94. **Negria rhabdothamnoides**, F. Muell. Fragm. vii, p. 152; viii, p. 281; ix, p. 78; Baill. in Assoc. Franç. pour l'Avanc. Sc. 1878, p. 646, t. 9; C. B. Clarke in DC. Monogr. Phanerog. v, pars i, p. 175.

Endemic.

A small tree attaining the height of eighteen feet, erroneously given as metres by Mr. Clarke.

The allied monotypic *Rhabdothamnus* is endemic in New Zealand. *Fieldia* is another monotypic genus of this order. This is restricted to Victoria and New South Wales. Besides these only two species of other genera of this order have been found in Australia—Queensland. The genus *Cyrtandra*, of which there are nearly a hundred species scattered over Polynesia, is not known to be represented in Australia.

XXXIX. BIGNONIACEAE.

95. **Tecoma austro-caledonica**, Bur. in Bull. Soc. Bot. Fr. ix (1862), p. 163.

Pandorea austro-caledonica, Seem. in Gard. Chron. 1870, p. 1085.

Tecoma australis, Moore Rep. p. 2, non R. Br.

New Caledonia.

Sir F. von Mueller (Fragm. ix, p. 77) also records *T. australis*, R. Br., from the island.

XL. ACANTHACEAE.

96. **Eranthemum variabile**, R. Br. Prod. p. 477; Benth. Fl. Austral. iv, p. 555; F. Muell. Fragm. ix, p. 78.

New South Wales, Queensland, and New Caledonia.

XLI. MYOPORACEAE.

97. **Myoporum insulare**, R. Br. Prodr. p. 516; Benth. Fl. Austral. v, p. 5 (sub *M. serrato*); F. Muell. Fragm. vi. p. 149; vii, p. 110; ix, p. 78; F. Muell. Myop. Pl. Frontisp. et t. 72; Gard. Chron. n. s. xxv (1886), pp. 492–3, fig. 92.

Myoporum acuminatum, Moore, Rep. p. 2, non R. Br.

Tasmania, West Australia, South Australia, Victoria, and New South Wales.

This and the Sandwich Islands species, *M. sandwicense*, A. Gr., are the only arboreous ones. The present one attains a height of forty feet, with a trunk of considerable thickness. A representation of it forms the frontispiece to Mueller's Lithograms of Myoporineous Plants.

XLII. VERBENACEAE.

98. **Avicennia officinalis**, L. Sp. Pl. p. 110; Benth. Fl. Austral. v, p. 69; Kirk, For. Fl. N. Zeal. t. 130; F. Muell. Fragm. ix, p. 78.

Avicennia tomentosa, Jacq. Enum. Pl. Carib. p. 25; Wall. Pl. As. Rar. t. 271; Wight, Ic. t. 1481.

Tropical and subtropical seashores throughout the world, though rare in Polynesia. It reaches New Zealand in the south and the Bermudas in the north.

XLIII. LABIATAE.

99. **Westringia rosmariniformis**, Sm. in Vet. Acad. Handl. Stockh. (1797) p. 171; Benth. Fl. Austral. v, p. 128; F. Muell. Fragm. ix, p. 78.

Westringia rosmarinacea, Andr. Bot. Rep. t. 214.

Tasmania, Victoria, New South Wales, and Queensland.

The genus *Westringia* is otherwise restricted to Australia and Tasmania, inhabiting all except the hotter parts.

XLIV. PLANTAGINEAE.

100. **Plantago varia**, R. Br. Prod. p. 424; Benth. Fl. Austral. v, p. 139; Turner, Forage Pl. Austral. p. 45; F. Muell. Fragm. ix, p. 78.

Throughout Australia.

An exceedingly variable species, of which nearly a dozen forms have been described as species.

XLV. NYCTAGINACEAE.

101. **Boerhaavia diffusa**, L. Fl. Zeyl. p. 4; Benth. Fl. Austral. v. p. 277; F. Muell. Key Vict. Pl. f. 44; F. Muell. Fragm. ix, p. 77.

Boerhaavia procumbens, Roxb. Fl. Ind. i, p. 146; Wight, Ic. t. 874.

Australia, Polynesia, and tropical and subtropical Asia and Africa.

102. **Pisonia umbellifera**, Seem. in Bonpl. x, p. 154; Fl. Vict. p. 195; Hillebr. Fl. Haw. p. 368; Drake, Fl. Polyn. Franç. p. 157.

Pisonia Brunoniana, Endl. Prodr. Fl. Norf. p. 43; Benth. Fl. Austral. v, p. 280; F. Muell. Fragm. ix, p. 77; Kirk, For. Fl. N. Zeal. t. 140.

Pisonia excelsa, Blume, Bijdr. p. 735; Hook. f. Fl. Brit. Ind. iv, p. 711.

New Zealand, Norfolk Island, New South Wales, and Queensland, throughout Polynesia, Malaya, and the Andaman Islands.

Authors by no means agree as to the limits of this species and the name it should bear. The synonyms are very numerous.

XLVI. CHENOPODIACEAE.

103. **Rhagodia Billardierii**, R. Br. Prod. p. 408; Benth. Fl. Austral. v, p. 152; F. Muell. Ic. Salsol. t. 21; F. Muell. Fragm. ix, p. 77.

Chenopodium baccatum, Labill. Pl. Nov. Holl. i, p. 71, t. 96.

Throughout Australia, in salt-marshes and on the coast.

104. **Atriplex cinereum**, Poir. Encyc. Suppl. i, p. 471; Benth. Fl. Austral. v, p. 171; Turner, Forage Pl. Austral.

p. 58; F. Muell. Ic. Salsol. t. 12; Hook. f. Handb. Fl. N. Zeal. p. 232; F. Muell. Fragm. ix, p. 77.

New Zealand, Tasmania, and Australia except the north; inhabiting the coast and salt-marshes.

XLVII. AMARANTACEAE.

105. **Achyranthes aspera**, L. Sp. Pl. p. 204; Benth. Fl. Austral. v, p. 246; Wight, Ic. t. 1777; F. Muell. Fragm. ix, p. 77.

Generally spread in warm countries.

XLVIII. POLYGONACEAE.

106. **Muehlenbeckia axillaris**, Walp. Ann. i, p. 552; Benth. Fl. Austral. v, p. 275; F. Muell. Fragm. ix, p. 77.

New Zealand, Tasmania, Victoria, and New South Wales.

XLIX. PIPERACEAE.

107. **Piper excelsum**, Forst. f. Prod. p. 5; Benth. Fl. Austral. vi, p. 204; Hook. f. Handb. Fl. N. Zeal. p. 254; F. Muell. Fragm. ix, p. 77.

New Zealand, Kermadec, Chatham, and Norfolk Islands, and Southern Polynesia.

108. **Peperomia reflexa**, A. Dietr. Sp. Pl. i, p. 180; Benth. Fl. Austral. vi, p. 207; Wight, Ic. t. 1923; F. Muell. Fragm. ix, p. 77.

Common in most warm countries.

109. **Peperomia Urvilleana**, A. Rich. in Urville, Voy. Bot. p. 356; F. Muell. Fragm. ix, p. 77; Hook. f. Handb. Fl. N. Zeal. p. 254.

New Zealand, Norfolk Island, and the Kermadecs.

Peperomia leptostachya, Hook. et Arn., is included in Prof. Tate's list, probably by mistake.

L. LAURINEAE.

110. **Cryptocarya triplinervis**, R. Br. Prod. p. 402; Benth. Fl. Austral. v, p. 297; F. Muell. Fragm. ix, p. 77.

Tetranthera, sp. Moore, Rep. p. 2.

New South Wales and Queensland.

LI. THYMELAEACEAE.

111. **Pimelea longifolia**, Banks et Sol. ex Wikstr. in Vet. Akad. Handl. Stockh. (1818) p. 280; Benth. Fl. Austral. vi, p. 7; F. Muell. Fragm. ix, p. 77; Hook. f. Handb. Fl. N. Zeal. p. 242.

Pimelea congesta, F. Muell. Fragm. viii, p. 9.

New Zealand, but confined to the main islands.

LII. SANTALACEAE.

112. **Exocarpus homalocladus**, F. Muell. Fragm. viii, p. 9; ix, p. 77; Benth. Fl. Austral. vi, p. 230.

Endemic.

The range of this singular genus is from New Zealand, Norfolk Island, and New Caledonia, throughout Australia and the Malay Archipelago to the Philippines, with an outlier in the Sandwich Islands. The supposed Madagascar species proves to be a member of the Leguminosae—*Phylloxylon*, Baill.

LIII. LORANTHACEAE.

113. **Viscum articulatum**, Burm. f. Fl. Ind. p. 311; Benth. Fl. Austral. iii, p. 396; F. Muell. Fragm. ix, p. 77.

Viscum moniliforme, Blume, Bijdr. p. 667; Wight, Ic. tt. 1018, 1019.

Viscum opuntioides, Moore, Rep. p. 2, non Linn.

New South Wales, Queensland, Polynesia, and tropical Asia.

LIV. EUPHORBIACEAE.

114. **Euphorbia Sparmanni**, Boiss. Cent. Euphorb. p. 5; Benth. Fl. Austral. vi, p. 46; Hemsl. in Journ. Linn. Soc. xxx, p. 191; F. Muell. Fragm. ix, p. 77.

New South Wales, Tonga, Elizabeth, and Pitcairn Islands.

115. **Hemicyclia australasica**, Muell. Arg. in DC. Prod. xv, II, p. 487; Benth. Fl. Austral. vi, p. 118

Hemicyclia sepiaria, F. Muell. Fragm. ix, p. 77.

New South Wales and Queensland.

116. **Baloghia lucida**, Endl. Prod. Fl. Norf. p. 84; Benth. Fl. Austral. vi, p. 148; Endl. Iconogr. tt. 122, 123; F. Muell. Census Austral. Pl. ed. II, p. 37; F. Muell. Fragm. ix, p. 77.

New Caledonia, Norfolk Island, New South Wales, and Queensland.

117. **Homalanthus Leschenaultianus**, A. Juss. Tent. Euphorb. p. 50, t. 16.

Carumbium populifolium, Reinw. in Blume, Cat. Hort. Buitenz. p. 105; Benth. Fl. Austral. vi, p. 150; F. Muell. Fragm. ix, p. 77.

Victoria, New South Wales, Queensland, Malaya, and Polynesia.

LV. URTICACEAE.

118. **Celtis amblyophylla**, F. Muell. Fragm. ix, pp. 76 et 77; Benth. Fl. Austral. vi, p. 157 (sub *C. paniculata*).

Endemic.

This is the cotton-wood of the settlers.

119. **Malaisia tortuosa**, Blanco, Fl. Filip. ed. I, p. 789; Benth. Fl. Austral. vi, p. 180; Vidal. Fl. For. Filip. t. 86, f. b; F. Muell. Fragm. ix, p. 77.

Caturus scandens, Lour. Fl. Cochinch. p. 612.

New South Wales, Queensland, North Australia, Western Polynesia, and Malaya.

120. **Ficus columnaris**, F. Muell. & C. Moore, in Proc. Acclim. Soc. Vict. iii (1874), p. 71; F. Muell. Fragm. vii. t. 61; ix, p. 77; Wils. Rep. p. 32.

Ficus rubiginosa, Desf.; Benth. Fl. Austral. vi, p. 168, pro parte.

Endemic.

Mr. Duff states that the fine old banyan trees are rapidly dying off, and he thinks there is a danger of the species becoming extinct in the island.

121. **Elatostema reticulatum**, Wedd. in Ann. Sci. Nat. ser. IV, i, p. 188; Benth. Fl. Austral. vi, p. 183; F. Muell. Fragm. ix, p. 77.

New South Wales and Queensland.

122. **Boehmeria calophleba**, C. Moore & F. Muell. in F. Muell. Fragm. viii, p. 11; ix, p. 77; Benth. Fl. Austral. vi, p. 184.

Endemic.

Allied to the Norfolk Island *B. australis*, Endl.

123. **Parietaria debilis**, Forst. f. Prod. p. 73; Benth. Fl. Austral. vi, p. 188; F. Muell. Fragm. ix, p. 77.

Widely spread in temperate and tropical regions of both hemispheres.

LVI. Orchideae.

124. **Dendrobium gracilicaule**, F. Muell. Fragm. i, p. 179; ix, p. 78; Benth. Fl. Austral. vi, p. 281; Bot. Mag. t. 7042.

Dendrobium elongatum, A. Cunn. in Bot. Reg. 1839, Misc. p. 33, non Lindl. Gen. et Sp. Orch. p. 77.

New South Wales and Queensland.

125. **Dendrobium Moorei**, F. Muell. Fragm. vii, p. 26; ix, p. 78; Benth. Fl. Austral. vi, p. 281; Fitzger. Austr. Orch. i, pt. 6.

Endemic.

126. **Bulbophyllum exiguum**, F. Muell. Fragm. ii, p. 72; ix, p. 78; Benth. Fl. Austral. vi, p. 288; Fitzger. Austr. Orch. ii, pt. 5.

New South Wales and Queensland.

Allied to *B. pygmaeum*, Lindl., an endemic, and the only New Zealand species.

127. **Cleisostoma erectum**, Fitzg. Orch. Austral. i, pt. iv.

Sarcochilus erectus, F. Muell. Census, ed. I, p. 111; ed. II, p. 187.

Endemic.

128. **Microtis porrifolia**, Spreng. Syst. iii, p. 713; Hook. f. Handb. Fl. N. Zeal. p. 266; Benth. Fl. Austral. vi, p. 347; Fitzger. Austral. Orch. v, pt. 2; F. Muell. Fragm. ix, p. 78; x, p. 65.

Tasmania, West Australia, South Australia, Victoria, New South Wales and Queensland, New Zealand and Polynesia.

LVII. AMARYLLIDACEAE.

129. **Crinum pedunculatum**, R. Br. Prod. p. 297; Benth. Fl. Austral. vi, p. 455; F. Muell. Fragm. ix, p. 78; Bull. Soc. Tosc. 1886, p. 267; Baker, Amaryl. p. 77; Bot. Reg. t. 52.

Crinum taitense, Redouté, Lil. t. 408.

South Australia, New South Wales, and Queensland, New Guinea, New Caledonia, and Fiji.

LVIII. IRIDEAE.

130. **Moraea Robinsoniana**, C. Moore & F. Muell. in F. Muell. Fragm. vii, p. 153; Benth. Fl. Austral. vi, p. 409; Bot. Mag. t. 7212.

Iris Robinsoniana, F. Muell. Fragm. vii, p. 153, tt. 63–64; ix, p. 78.

This is apparently endemic, but Mueller (Fragm. viii, p. 281) states that 'Cl. Hill hanc plantam e montibus Bellenden Kerii indicat, unde autem nondum exemplaria habeo.'

LIX. LILIACEAE.

131. **Smilax australis**, R. Br. Prod. p. 293; Benth. Fl. Austral. vii, p. 7; F. Muell. Key Vict. Pl. t. 117; F. Muell. Fragm. ix, p. 78.

Smilax elliptica et *latifolia*, R. Br. Prod. p. 293.

Eastern Australia from Victoria northwards to the islands of the Gulf of Carpentaria.

A. de Candolle (Monogr. Phaner. i, p. 64) records the Polynesian *Smilax purpurata*, Forst., from Howe Island.

132. **Geitonoplesium cymosum**, A. Cunn. in Bot. Mag. t. 3131; Benth. Fl. Austral. vii, p. 19.

Victoria, New South Wales, and Queensland, Norfolk Island, South Pacific Islands, and Borneo.

A monotype.

133. **Dianella caerulea**, Sims, Bot. Mag. t. 505; Benth. Fl. Austral. vii, p. 16.

New South Wales, Queensland, and North Australia.

There is no specimen of this in the Kew Herbarium.

LX. COMMELINACEAE.

134. **Commelina cyanea**, R. Br. Prod. p. 269; Benth. Fl. Austral. vii, p. 84.

C. communis, Muell. Fragm. viii, p. 59; ix, p. 78, non Linn.; Clarke, Comm. & Cyrt. Beng. t. 1, et in DC. Monogr. Phanerog. iii, p. 147.

Eastern Australia and New Caledonia.

LXI. FLAGELLARIACEAE.

135. **Flagellaria indica**, L. Sp. Pl. p. 333; Benth. Fl. Austral. vii, p. 10; Red. Lil. v, t. 257; Hemsl. Rep. Voy. *Chall.* Bot. i, pt. 3, p. 202; F. Muell. Fragm. ix, p. 78.

Australasia, Western Polynesia, tropical Asia and Africa, chiefly on the seashores.

The specimen from the island in the Kew Herbarium, collected by Milne, consists of leaves only, remarkable for their length, being about eighteen inches long.

The only other species of the genus, *F. gigantea*, Hook. f. (Hooker's Ic. Pl. t. 1429), inhabits Samoa and Fiji.

LXII. JUNCACEAE.

136. **Juncus maritimus**, Lam. Encyc. iii, p. 264; Benth. Fl. Austral. vii, p. 130; Engl. Bot. ed. III, t. 1559; Buchen. Abh. Nat. Ver. Bremen, iv, t. 4; F. Muell. Fragm. ix, p. 78.

Temperate and subtropical regions of the eastern hemisphere.

137. **Luzula longiflora**, Benth. Fl. Austral. vii, p. 123; F. Muell. Census Austral. Pl. ed. II, p. 206.

Luzula campestris, F. Muell. Fragm. ix, p. 78, non DC.

Endemic.

LXIII. PALMAE.

138. **Hedyscepe Canterburyana**, Wendl. et Drude in Linnaea, xxxix (1875), pp. 178, 189, et 203, t. 1, fig. 4.

Kentia Canterburyana, F. Muell. Fragm. vii, p. 101, t. 62; viii, p. 234; ix, p. 78; Benth. Fl. Austral. vii, p. 138; Duff in Wils. Rep. p. 31.

Endemic.

The Umbrella Palm of the settlers.

139. **Clinostigma Mooreanum**, F. Muell. Fragm. viii, p. 235; Benth. Fl. Austral. vii, p. 139; Wendl. et Drude in Linnaea, xxxix (1875), pp. 185 et 218, t. 2, fig. 5.

Clinostigma Moorei, F. Muell. Fragm. ix, p. 78; Duff in Wils. Rep. p. 31.

Kentia Mooreana, F. Muell. Fragm. vii, p. 101; viii, p. 234.

Endemic.

A dwarf Palm not more than six feet high, restricted to the summits of the mountains.

140. **Howea Belmoreana**, Becc. Malesia, i (1877), p. 66.

Kentia Belmoreana, F. Muell. Fragm. vii, p. 99, t. 61; viii, p. 234; ix, p. 78; Benth. Fl. Austral. vii, p. 137; Duff in Wils. Rep. p. 30.

Grisebachia Belmoreana, Wendl. et Drude in Linnaea, xxxix (1875), p. 202, t. 4, fig. 1.

Endemic.

The Curly Palm of the settlers.

141. **Howea Forsteriana**, Becc. Malesia, i (1877), p. 66.

Kentia Forsteriana, F. Muell. ex H. Wendl. in Kerch. Palm. p. 248; F. Muell. Fragm. vii, t. 61; ix, p. 78; Duff in Wils. Rep. p. 30.

Grisebachia Forsteriana, Wendl. et Drude in Linnaea, xxxix (1875), p. 203, t. 4, fig. 2.

Endemic.

The Thatch Palm of the settlers.

Mr. Duff gives the following particulars of the Palms, which have been confused in the young state:—

'Both palms [*Howea Forsteriana* and *H. Belmoreana*] flower exactly alike, i.e. they produce their flower-spikes generally from the axils of the lowest row of leaves, but occasionally young undeveloped flower-spikes spring from the axils of the leaves above them, and their ripe seeds [fruits] are always emitted from immediately under the leaves. The seeds [fruits] of the curly palm are oval and a greenish-yellow colour when ripe, whilst those of the thatch palm taper to a point at both ends and are dark crimson when mature.'

'The curly palm is the most abundant and wide-spread species, as it extends from the beach to an elevation of about 1,200 feet on the

sides of Mounts Gower and Lidgbird, whilst the thatch palm is confined chiefly to the beach, not being found farther up the mountains than from 300 to 400 feet.'

'The fronds and pinnae of the curly palm are recurved at the apex, and the pinnae are nearly erect at the base, the thatch palm having less recurved darker green fronds and broader, pendulous pinnae, which distinctions are observable even in the small seedling plants.'

'The chief specific distinctions, however, between these two palms are as follows:—The curly palm bears its flower-spikes singly, which average 5 to 6 feet in length, and those of the thatch palm consist of five spikes in a row, united together at the base, of an average length of 3 to 4 feet.'

LXIV. PANDANACEAE.

142. **Pandanus Forsteri**, C. Moore & F. Muell. in F. Muell. Fragm. viii, p. 220; ix, p. 78; Benth. Fl. Austral. vii, p. 149; Duff in Wils. Rep. p. 32.

Endemic.

143. **Pandanus** (species imperfecte cognita), Moore, Rep. p. 2; Duff in Wils. Rep. p. 29.

Endemic.

'The mountain *Pandanus* is evidently another undescribed plant, differing from *Pandanus Forsteriana* in having smaller and more numerous branches; shorter, more undulating, and narrower leaves; cones 6 to 8 inches long, or less than half the size of those of *P. Forsteriana*; height, 20 to 30 feet, with a diameter across the branches of 20 feet; the stems are about 6 inches in diameter, and aerial roots are produced on the branches, a peculiarity rarely seen in *Pandanus Forsteriana*.'—DUFF.

LXIV A. NAIADACEAE.

143 A. **Halophila ovata**, Gaud. in Freyc. Voy., Bot. t. 40, f. 1; F. Muell. Fragm. viii, p. 219.

Halophila ovalis, Hook, f. Fl. Tasm. ii, p. 45; Benth. Fl. Austral. vii, p. 182.

Coasts of Tasmania, Victoria, and New South Wales to North Australia; also in the Pacific and Indian Oceans.

LXV. CYPERACEAE[1].

144. **Cyperus haematodes**, Endl. Prodr. Fl. Norf. p. 22; Benth. Fl. Austral. vii, p. 285; F. Muell. Census Austral. Pl. ed. II, p 211.

Cyperus congestus, forma *gigantea*, F. Muell. Fragm. viii, p. 269.

Cyperus congestus, F. Muell. Fragm. ix, p. 78 non Vahl.

Norfolk Island and Queensland. There is also a specimen in the Kew Herbarium labelled 'Nouvelle Hollande, Verreaux.'

144 A. **Cladium insulare**, Benth. Fl. Austral. vii, p. 123.

Gahnia insularis, F. Muell. Census Austral. Pl. ed. II, p. 216.

Endemic.

145. **Scirpus nodosus**, Rottb. Desc. et Ic. Pl. Nov. p. 52, t. 8, f. 3; Progr. p. 24; Benth. Fl. Austral. vii, p. 331; Hemsl. Rep. Voy. *Chall.* Bot. i, 2, p. 87; F. Muell. Fragm. ix, p. 78.

Isolepis nodosa, R. Br. Prod. p. 221; Rich. Bot. Voy. Astrol. N. Zél. t. 18.

South temperate and subtropical regions, generally, including the remote islands of St. Helena, St. Paul, and Amsterdam.

146. **Gahnia xanthocarpa**, Hook. f. Handb. New Zeal. Fl. p. 306; Benth. Fl. Austral. vii, p. 418.

Cladium xanthocarpum, F. Muell. Fragm. ix, p. 13, et p. 78.

Lampocarya, sp. C. Moore in Trans. Roy. Soc. N. S. Wales, v, p. 32.

New Zealand, in the northern island only.

147. **Uncinia filiformis**, Colenso; Hook. f. Fl. N. Zeal. i, p. 286; Handb. Fl. N. Zeal. p. 310.

Uncinia debilior, F. Muell. Fragm. viii, p. 151; ix, p. 78; Benth. Fl. Austral. vii, p. 435.

New Zealand.

The Howe Island plant is reduced to *U. filiformis*, Boott, on the authority of Mr. C. B. Clarke.

148. **Carex breviculmis**, R. Br. Prod. p. 242; Benth. Fl.

[1] I am indebted to Mr. C. B. Clarke for kindly verifying the nomenclature of the members of this order.—W. B. H.

Austral. vii, p. 445; Hook. f. Fl. N. Zeal. t. 63; F. Muell. Fragm. ix, p. 78.

New Zealand, Tasmania, Australia except the west, and Malaya to the Himalayas.

149. **Carex gracilis**, R. Br. Prod. p. 242; Benth. Fl. Austral. vii, p. 442; Boott. Ill. Car. i, p. 59, tt. 154–155; F. Muell. Fragm. ix, p. 78.

Queensland and New South Wales.

LXVI. GRAMINEAE.

150. **Panicum sanguinale**, L. Sp. Pl. p. 57; Benth. Fl. Austral. vii, p. 469; Trin. Spec. Gram. t. 93; Agric. Gaz. N. S. Wales, ii, t. 21.

Now generally dispersed in warm countries, but commonly as a weed.

151. **Oplismenus compositus**, Beauv. Agrost. p. 54; Benth. Fl. Austral. vii, p. 491; Agric. Gaz. N. S. Wales, ii, t. 41.

Panicum compositum, L. Sp. Pl. p. 57; Trin. Spec. Gram. ii, tt. 187–188, 190; F. Muell. Fragm. viii, p. 199; ix, p. 78.

Generally dispersed in warm countries.

This includes *O. setarius*, Roem. et Schult, *O. undulatifolius*, Beauv., and a host of other synonyms.

152. **Phragmites communis**, Trin.; Benth. Fl. Austral. vii, p. 636; Hook. f. Handb. N. Zeal. Fl. p. 746.

Of world-wide range.

153. **Spinifex hirsutus**, Labill. Nov. Holl. Pl. ii, p. 81, tt. 230–31; Benth. Fl. Austral. vii, p. 503; F. Muell. Fragm. ix, p. 78; Buchan. Man. Gr. N. Zeal. tt. 8–9; Agric. Gaz. N. S. Wales, v, 1894, p. 835.

S. sericeus, R. Br. Prod. p. 198.

New Zealand, New Caledonia, Australia, and Polynesia.

154. **Stipa micrantha**, Cav. Ic. et Descr. Pl. v, p. 42, t. 467; Benth. Fl. Austral. vii, p. 566; F. Muell. Fragm. ix, p. 78.

New Zealand and Australia, except the north.

155. **Sporobolus indicus**, R. Br. Prod. p. 170; Benth. Fl. Austral. vii, p. 622; Bail. Gr. Queensl. i; Agric. Gaz. N. S. Wales, ii, t. 29; v, p. 389.

S. elongatus, R. Br. Prod. p. 170; F. Muell. Fragm. ix, p. 78.

Vilfa tenacissima, Trin. Spec. Gram. t. 60; Buchan. Man. Gr. N. Zeal. t. 18.

Generally dispersed in warm countries, and extending to some temperate regions, including New Zealand.

156. **Deyeuxia Forsteri**, Kunth, Rév. Gram. i, p. 77; Benth. Fl. Austral. vii, p. 579.

Agrostis Solandri, F. Muell. Veg. Chat. Isl. p. 60.

Agrostis aemula, R. Br. Prod. p. 172; Buchan. Man. Gr. N. Zeal. t. 21.

New Zealand, Tasmania, and Australia except the north.

157. **Dichelachne crinita**, Hook. f. Fl. New Zeal. i, p. 293; Benth. Fl. Austral. vii, p. 574; Bail. Gr. Queensl. i; Buchan. Man. Gr. N. Zeal. t. 15.

Anthoxanthum crinitum, L. f. Suppl. p. 90; Labill. Pl. Nov. Holl. ii, p. 115, t. 263.

New Zealand, Tasmania, and Australia except the north.

158. **Cynodon Dactylon**, Pers. Syn. Pl. i, p. 85; Benth. Fl. Austral. vii, p. 609; Reichb. Ic. Fl. Germ. t. 26; Agric. Gaz. N. S. Wales, ii, 1891, t. 24; Mart. Fl. Bras. ii, III, t. 21, f. 3; F. Muell. Fragm. ix, p. 78.

Cosmopolitan in warm countries, and extending to some temperate regions. Often cultivated under the name of Bermuda grass.

159. **Chloris Pumilio**, R. Br. Prod. p. 186; Benth. Fl. Austral. vii, p. 611; Bail. Gr. Queensl. i; C. Moore, Rep. p. 4.

There is no specimen of this very distinct grass in the Kew Herbarium from the island, nor is it in Mueller's list, and it is included on the authority of Moore's list. It is a native of Queensland and North Australia.

160. **Poa caespitosa**, Forst. f. Fl. Ins. Austral. Prod. p. 89; Benth. Fl. Austral. vii, p. 651; F. Muell. Fragm. ix, p. 78; Spreng. in Mem. Acad. Petersb. ii (1807–8), p. 302, t. 8; Agric. Gaz. N. S. Wales, iv, p. 524.

Poa australis, R. Br. Prod. p. 179; Buchan. Man. Gr. N. Zeal. t. 47.

New Zealand, Tasmania, and Australia except the north.

161. **Agropyrum scabrum**, Beauv. Agrost. p. 102; Benth. Fl. Austral. vii, p. 665; Agric. Gaz. N. S. Wales, ii, t. 16.

Triticum scabrum, R. Br. Prod. p. 178.

Festuca Billardierii, Steud. Syn. Pl. Gram. p. 304; F. Muell. Fragm. ix, p. 78.

Festuca scabra, Labill. Pl. Nov. Holl. i, p. 22, t. 26.

New Zealand, Norfolk Island, Tasmania, and Australia except the north.

LXVII. Lycopodiaceae.

162. **Lycopodium varium**, R. Br. Prod. p. 165; Benth. Fl. Austral. vii, p. 674; Hook. & Grev. Ic. Filic. t. 112.

Lycopodium Billardieri, Spring, Monogr. Lycopod. i, p. 57; ii, p. 24.

New Zealand, Tasmania, Victoria, Polynesia, and South Africa.

163. **Psilotum triquetrum**, Sw. Syn. Filic. p. 187; Benth. Fl. Austral. vii, p. 681; Hook. Gen. Filic. t. 87; Hook. Filic. Exot. t. 63; F. Muell. Fragm. x, p. 118; Hemsl. Rep. Voy. *Chall.* Bot. i, 3, p. 258.

Asia, Africa, America, and Australia, and the most remote islands of Polynesia.

Mueller mentions having specimens of this from the island, three feet long.

164. **Tmesipteris tannensis**, Bernh. in Schrad. Journ. ii (1800), p. 131, t. 2, f. 5; Benth. Fl. Austral. vii, p. 680; Labill. Pl. Nov. Holl. ii, p. 105, t. 252; F. Muell. Fragm. ix, p. 78.

Tmesipteris Forsteri, Endl.; Hook. f. Handb. Fl. N. Zeal. p. 391.

New Zealand, Norfolk Island, Tasmania, Eastern Australia, and Polynesia.

LXVIII. Selaginellaceae.

165. **Selaginella uliginosa**, Spring, Monogr. Lycopod. ii, p. 60; Benth. Fl. Austral. vii, p. 678.

Lycopodium uliginosum, Labill. Pl. Nov. Holl. ii, p. 104, t. 251.

Tasmania, and Australia except the north.

LXIX. Filices.

166. **Cyathea brevipinna**, Baker, in Benth. Fl. Austral. vii, p. 709.

Endemic.

This is most likely the plant referred in Mueller's list (Fragm. ix, p. 78) to *C. medullaris*, Sw.

167. **Cyathea Macarthurii**, F. Muell. Fragm. vii, p. 177; Benth. Fl. Austral. vii, p. 708.

Cyathea Moorei, Hook. & Baker, Synop. Fil. p. 453.

Hemitelia Macarthurii, F. Muell. Fragm. viii, p. 176; ix, p. 78.

Endemic.

168. **Hemitelia Moorei**, Baker, in Gard. Chron. (1872), p. 252; Benth. Fl. Austral. vii, p. 709; F. Muell. Fragm. ix, p. 78.

Endemic.

169. **Alsophila australis**, R. Br. Prod. p. 158, var.? **nigrescens**; Benth. Fl. Austral. vii, p. 710.

Alsophila excelsa, R. Br. in Endl. Prod. Fl. Norf. p. 16; Hook. Spec. Filic. i. p. 49, t. 18 a; Gard. Chron. 1871, p. 610; F. Muell. Fragm. ix, p. 78.

Tasmania, South and East Australia, and Norfolk Island.

170. **Dicksonia nephrodioides**, F. Muell. Census, ed. I, p. 137; F. Muell. Fragm. ix, p. 78.

Deparia nephrodioides, Baker, in Gard. Chron. (1872), p. 253; Benth. Fl. Austral. vii, p. 714; Hook. Ic. Pl. t. 1608.

Davallia nephrodioides, F. Muell. Fragm. x, p. 104.

Endemic.

171. **Hymenophyllum flabellatum**. Labill. Pl. Nov. Holl. ii, p. 101, t. 250; Benth. Fl. Austral. vii, p. 705.

Hymenophyllum nitens, R. Br. Prod. p. 159; Hook. & Grev. Ic. Filic. t. 197.

New Zealand, Tasmania, Eastern Australia, and Polynesia.

172. **Hymenophyllum minimum**, A. Rich. Fl. Nouv. Zél. p. 19, t. 14, f. 2; Benth. Fl. Austral. vii, p. 706.

New Zealand and Auckland Isles.

173. **Hymenophyllum multifidum**, Sw. Syn. Filic. pp. 149,

378; Benth. Fl. Austral. vii, p. 707; Hook. & Grev. Ic. Filic. t. 167.

New Zealand and Southern Polynesia.

174. **Hymenophyllum pumilum**, C. Moore, in Hook. & Baker, Synop. Fil. p. 464; Benth. Fl. Austral. vii, p. 706; C. Moore, Handb. Fl. N. S. W. p. 504.

Hymenophyllum Moorei, Baker, in Hook. & Baker, Synop. Fil. p. 464.

New South Wales.

175. **Hymenophyllum tunbridgense**, Sm. in Roem. Archiv, i, p. 56; Benth. Fl. Austral. vii, p. 706; Bedd. Ferns S. India, t. 265; F. Muell. Fragm. ix, p. 78.

Hymenophyllum cupressiforme, Labill. Pl. Nov. Holl. ii, p. 102, t. 250.

Almost cosmopolitan in temperate and subtropical regions.

176. **Trichomanes apiifolium**, Presl, Hymenophyllaceae, p. 44; Benth. Fl. Austral. vii, p. 703.

Trichomanes meifolium, Bory, in Willd. Sp. Pl. v, p. 509; F. Muell. Fragm. ix, p. 78.

Trichomanes polyanthos, Hook. Ic. Pl. t. 703.

Trichomanes Bauerianum, Endl. Prodr. Fl. Norf. p. 17.

Eastern Australia, Norfolk Island, Polynesia, and Malaya.

177. **Davallia dubia**, R. Br. Prod. p. 157; Benth. Fl. Austral. vii, p. 716.

Dicksonia dubia, Gaudich. in Freyc. Voy. Bot. p. 367; Hook. Spec. Filic. i, p. 71, t. 24.

Tasmania and South and East Australia.

178. **Adiantum aethiopicum**, L. Syst. Nat. ed. X, n. 15; Benth. Fl. Austral. vii, p. 724; Bedd. Ferns S. India, t. 5; F. Muell. Fragm. ix, p. 78.

Adiantum trigonum, Labill. Pl. Nov. Holl. ii, p. 99, t. 248.

Widely dispersed in tropical and temperate regions of the Old World and Western America.

179. **Adiantum hispidulum**, Sw. Syn. Filic. pp. 124, 321; Benth. Fl. Austral. vii, p. 725; Bedd. Ferns S. Ind. t. 3; F. Muell. Fragm. ix, p. 78.

New Zealand, Australasia, Polynesia, Asia, and Africa.

180. **Hypolepis tenuifolia**, Bernh. in Schrad. Journ. ii (1800), p. 24; Benth. Fl. Austral. vii, p. 726; Hook. Spec. Filic. ii, p. 60, tt. 89-90; F. Muell. Fragm. ix, p. 78.

New Zealand, Eastern Australia, Polynesia, and Malaya.

181. **Cheilanthes tenuifolia**, Sw. Syn. Filic. p. 126; Benth. Fl. Austral. vii, p. 726; Hook. Spec. Filic. ii, p. 82, t. 87; F. Muell. Fragm. ix, p. 78.

New Zealand, throughout Australia, Malaya, and India.

182. **Pteris aquilina**, L. Sp. Pl. p. 1075, var. **esculenta**; Benth. Fl. Austral. vii, p. 731; Bedd. Ferns S. Ind. t. 42; F. Muell. Fragm. ix, p. 78.

Pteris esculenta, Forst. f. Prod. p. 79; Labill. Pl. Nov. Holl. ii, p. 95, t. 244.

The typical form is almost cosmopolitan, but the variety *esculenta* is confined to the southern hemisphere.

183. **Pteris comans**, Forst. f. Prod. p. 79; Benth. Fl. Austral. vii, p. 733; Hemsl. Rep. Voy. *Chall.* Bot. i, 3, p. 70; F. Muell. Fragm. ix, p. 78.

Pteris Endlicheriana, Agardh. Sp. Gen. Pterid. p. 66; Hook. Ic. Pl. t. 973.

New Zealand, Tasmania, South and East Australia, and Polynesia.

184. **Pteris falcata**, R. Br. Prod. p. 154; Benth. Fl. Austral. vii, p. 729; F. Muell. Fragm. ix, p. 78.

Pellaea seticaulis, Hook. Ic. Pl. t. 207.

Platyloma falcatum, J. Sm. Cult. Ferns, p. 32; Bedd. Ferns S. Ind. t. 22.

New Zealand, Tasmania, South and East Australia, Malaya, and India.

185. **Pteris incisa**, Thunb. Prod. Fl. Cap. p. 171; Benth. Fl. Austral. vii, p. 732; Hemsl. Rep. Voy. *Chall.* Bot. i. 3, p. 70; F. Muell. Fragm. ix, p. 78.

Pteris vespertilionis, Labill. Pl. Nov. Holl. ii, p. 96, t. 245.

Tropical regions generally and southern extratropical countries.

186. **Pteris tremula**, R. Br. Prod. p. 154; Benth. Fl. Austral. vii, p. 731; Hook. Spec. Filic. ii, p. 174, t. 120.

Pteris arguta, F. Muell. Fragm. ix, p. 78, vix Ait.

New Zealand, Tasmania, South and East Australia, Norfolk Island, and Western Polynesia.

187. **Lomaria attenuata**, Willd. Sp. Pl. v, 290; Benth. Fl. Austral. vii, p. 736; Hemsl. Rep. Voy. *Chall.* Bot. i, 3, p. 71.

Kermadec Islands, Polynesia, Mascarene Islands, South Africa, and South America, but not known either from Australia or New Zealand.

188. **Lomaria capensis**, Willd. Sp. Pl. v, p. 291; Benth. Fl. Austral. vii, p. 737; Hemsl. Rep. Voy. *Chall.* Bot. i, 3, p. 73; F. Muell. Fragm. ix, p. 78.

Lomaria procera, Spreng. Syst. Veg. iv, p. 65; Hook. Ic. Pl. t. 427; Gard. Ferns, t. 53.

Very widely dispersed in tropical and southern extratropical regions.

189. **Lomaria Fullagari**, F. Muell. Fragm. viii p. 157; ix, p. 78; Benth. Fl. Austral. vii, p. 737.

Lomaria auriculata, Baker, Synop. Fil. p. 481.

Endemic.

190. **Doodia aspera**, R. Br. Prod. p. 151; Benth. Fl. Austral. vii, p. 741; Hook. Exot. Fl. t. 8.

Woodwardia aspera, Mett.; Bailey, Queensland Ferns, p. 27.

Victoria, New South Wales, and Queensland.

191. **Asplenium falcatum**, Lam. Encyc. Meth. i, p. 303; Benth. Fl. Austral. vii, p. 746; Bedd. Ferns S. Ind. t. 141; F. Muell. Fragm. ix, p. 78.

New Zealand, Eastern Australia, Polynesia, tropical Asia and Africa.

192. **Asplenium melanochlamys**, Hook. Sp. Filic. iii, p. 259; Synop. Fil. p. 239; Benth. Fl. Austral. vii, p. 751; F. Muell. Fragm. ix, p. 78.

Endemic.

193. **Asplenium obtusatum**, Forst.; Hook. Syn. Fil. p. 207; Hook. Fil. Exot. t. 46; Benth. Fl. Austral. vii, p. 747 (var. **incisum**).

Asplenium marinum cum pluribus varietatibus, F. Muell. Fragm. ix, p. 78.

Tasmania, New South Wales, New Zealand, Norfolk Island, and extratropical South America.

194. **Asplenium Nidus**, L. Sp. Pl. p. 1079; Benth. Fl. Austral. vii, p. 744; Hemsl. Rep. Voy. *Chall.* Bot. i, 3, p. 253; Bot. Mag. t. 3101; F. Muell. Fragm. ix, p. 78.

Asplenium australasicum, Hook. Filic. Exot. t. 88.

Widely spread in the warmer parts of the Old World, including Eastern Australia and Polynesia, eastward to Mangaia, Elizabeth Island, Tahiti, and the Sandwich Islands.

195. **Asplenium pteridioides**, Baker, Syn. Filic. p. 488; Benth. Fl. Austral. vii, p. 749; Hook. Ic. Pl. t. 1649.

Endemic.

196. **Aspidium apicale**, Baker, in Benth. Fl. Austral. vii, p. 758.

Endemic.

Mueller's list (Fragm. ix, p. 78) includes the widely-spread *A. aculeatum*, but I have seen no specimen.

197. **Aspidium capense**, Willd. Sp. Pl. v, p. 267; Benth. Fl. Austral. vii, p. 758; Hemsl. Rep. Voy. *Chall.* Bot. i, 3, p. 75.

Aspidium coriaceum, Sw. Syn. Fil. p. 57; Hook. Sp. Fil. iv, p. 32.

Widely spread in tropical and southern extratropical regions, including the remote islands of Tristan d'Acunha and St. Paul.

198. **Aspidium cordifolium**, Sw. Syn. Filic. p. 45; Benth. Fl. Austral. vii, p. 754.

Aspidium tuberosum, Bory; F. Muell. Fragm. v, p. 136; ix, p. 78.

Nephrolepis tuberosa, Presl, Tent. Pterid. p. 79; Bedd. Ferns S. Ind. t. 92; Hook. f. Handb. Fl. N. Zeal. p. 379.

Northern Island New Zealand, New South Wales, Queensland, and generally spread in the tropics.

199. **Aspidium molle**, Sw. Syn. Filic. p. 46; Benth. Fl. Austral. vii, p. 756.

Polypodium molle, Jacq. Ic. Rar. t. 640.

Nephrodium molle, R. Br. Prod. p. 149; Bedd. Ferns S. Ind. t. 84; Hemsl. Rep. Voy. *Chall.* Bot. i, 3, p. 254.

Generally dispersed in warm countries.

200. **Polypodium australe**, Mett. Filic. Hort. Bot. Lips. (1856) p. 36; Benth. Fl. Austral. vii, p. 762; Hook. f. Handb. Fl. N. Zeal. p. 380; Hemsl. Rep. Voy. *Chall.* Bot. i, 2, p. 196; F. Muell. Fragm. ix, p. 78.

Extratropical South America, Tasmania, Victoria, New South Wales, Queensland, New Zealand, and Chatham, Lord Auckland, Marion, Amsterdam, and Tristan d'Acunha Islands.

201. **Polypodium confluens**, R. Br. Prod. p. 146; Benth. Fl. Austral. vii, p. 767; Fée, Fil. Bras. t. 26.

Eastern Australia, Norfolk Island, and New Caledonia.

Mueller includes in his list (Fragm. ix, p. 78) *P. serpens*, Forst., a species common to Eastern Australia, New Zealand, and Polynesia, which has been confused with *P. confluens*.

202. **Polypodium Hookeri**, Brackenr. Bot. Wilk. Exped. Filic. p. 4; Benth. Fl. Austral. vii, p. 763; F. Muell. Fragm. ix, p. 78.

Polypodium setigerum, Hook. & Arn. Bot. Beech. Voy. p. 103, t. 21.

Eastern Australia, the Philippine and Sandwich Islands.

203. **Polypodium punctatum**, Thunb. Fl. Jap. p. 337; Benth. Fl. Austral. vii, p. 764; Hillebr. Fl. Haw. p. 553.

Polypodium rugulosum, Labill. Pl. Nov. Holl. ii, p. 92, t. 241; Bedd. Ferns S. Ind. t. 170; Hook. f. Handb. Fl. N. Zeal. p. 381.

Widely spread in the southern hemisphere, including the Chatham, Lord Auckland, Juan Fernandez, St. Helena, and Tristan d'Acunha Islands, and extending northward to Japan.

204. **Polypodium pustulatum**, Forst. f. Prod. p. 81 ; Benth. Fl. Austral. vii, p. 769 ; Hook. f. Handb. Fl. N. Zeal. p. 382.
Polypodium scandens, Labill. Pl. Nov. Holl. ii, p. 91, t. 240.
Polypodium Billardieri, R. Br. ; Muell. Fragm. ix, p. 78.

New Zealand, Norfolk Island, Tasmania, and Eastern Australia.

205. **Polypodium tenellum**, Forst. f. Prod. p. 440 ; Benth. Fl. Austral. vii, p. 764 ; F. Muell. Fragm. ix, p. 78.
Arthropteris tenella, J. Sm. in Hook. f. Fl. N. Zeal. t. 82.

New Zealand, Norfolk Island, New Caledonia, and Eastern Australia.

206. **Notholaena distans**, R. Br. Prod. p. 146 ; Benth. Fl. Austral. vii, p. 774 ; Hook. Ic. Pl. t. 980.

New Zealand, Norfolk Island, New Caledonia, Australia except the north, and Polynesia.

207. **Platycerium alcicorne**, Desv. in Mém. Soc. Linn. Par. vi (1827), p. 213 ; Benth. Fl. Austral. vii, p. 780; Gard. Chron. 1872, p. 511 ; F. Muell. Fragm. ix, p. 78.
Acrostichum alcicorne, Sw. Syn. Filic. p. 17 ; Bot. Reg. t. 262.

Eastern Australia and the Mascarene Islands.

208. **Todea Moorei**, Baker, in Journ. Bot. xi (1873), p. 16 ; Benth. Fl. Austral. vii, p. 700 ; F. Muell. Fragm. ix, p. 78.

Endemic.

209. **Marattia fraxinea**, Sm. Ic. Ined. t. 48 ; Benth. Fl. Austral. vii, p. 695 ; Bedd. Ferns S. Ind. t. 79.
Marattia salicina, Sm. ; F. Muell. Fragm. ix, p. 78 ; x, p. 118.

New Zealand, Eastern Australia, Polynesia, and widely dispersed in tropical Asia and Africa.

TABLE SHOWING THE DISTRIBUTION OF THE GENERA REPRESENTED IN THE ISLAND.

	Australia.	New Zealand.	Norfolk Island.	Polynesia.	Malayan Archipelago.	Of wider range.
1. Clematis	1	1	1	1	1	1
2. Drimys	1	1		1	1	1
3. Stephania	1			1	1	1
4. Lepidium	1	1		1		1
5. Hymenanthera	1	1	1			
6. Xylosma	1			1	1	1
7. Pittosporum	1	1	1	1	1	1
8. Calophyllum	1			1	1	1
9. Hibiscus	1	1	1	1	1	1
10. Lagunaria	1		1			
11. Pelargonium	1	1				1
12. Oxalis	1	1	1	1	1	1
13. Melicope	1	1		1		
14. Evodia	1			1	1	1
15. Zanthoxylum	1		1		1	1
16. Acronychia	1	1		1	1	1
17. Dysoxylum	1	1		1	1	1
18. Elaeodendron	1		1	1	1	1
19. Cupania	1			1	1	1
20. Atalaya	1				1	
21. Dodonaea	1	1	1	1	1	1
22. Carmichaelia		1				
23. Mucuna	1			1	1	1
24. Canavalia	1		1	1	1	1
25. Vigna	1			1	1	1
26. Sophora	1	1		1	1	1
27. Caesalpinia	1		1	1	1	1
28. Colmeiroa						
29. Leptospermum	1	1			1	1
30. Melaleuca	1				1	1
31. Acicalyptus				1		
32. Metrosideros	1	1		1	1	1
33. Passiflora	1	1	1	1	1	1
34. Sicyos	1	1	1	1	1	1
35. Mesembryanthemum	1	1	1			1
36. Tetragonia	1	1	1	1		1
37. Sesuvium	1			1	1	1
38. Hydrocotyle	1	1		1	1	1
39. Apium	1	1	1	1	1	1
40. Panax	1	1		1	1	1
	37	23	16	30	30	33

	Australia.	New Zealand.	Norfolk Island.	Polynesia.	Malayan Archipelago.	Of wider range.
	37	23	16	30	30	33
41. Randia	1			1	1	1
42. Psychotria	1			1	1	1
43. Coprosma	1	1	1	1	1	1
44. Brachycome	1	1				
45. Olearia	1	1				
46. Gnaphalium	1	1	1	1	1	1
47. Cassinia	1	1		1		
48. Wedelia	1		1	1	1	1
49. Bidens	1	1	1	1	1	1
50. Erechthites	1	1			1	1
51. Senecio	1	1	1	1	1	1
52. Scaevola	1	1		1	1	1
53. Lobelia	1	1	1	1	1	1
54. Wahlenbergia	1	1		1	1	1
55. Leucopogon	1	1		1	1	1
56. Dracophyllum	1	1		1		
57. Myrsine	1	1	1	1	1	1
58. Aegiceras	1			1	1	1
59. Sideroxylon	1	1		1	1	1
60. Symplocos	1			1	1	1
61. Jasminum	1		1	1	1	1
62. Notelaea	1					
63. Olea	1	1	1	1	1	1
64. Alyxia	1		1	1	1	1
65. Ochrosia	1			1	1	1
66. Lyonsia	1					
67. Vincetoxicum	1				1	1
68. Tylophora	1			1	1	1
69. Marsdenia	1				1	1
70. Geniostoma	1	1		1	1	1
71. Solanum	1	1	1	1	1	1
72. Ipomoea	1	1	1	1	1	1
73. Calystegia	1	1	1	1		1
74. Negria						
75. Tecoma	1			1	1	1
76. Eranthemum	1			1	1	1
77. Myoporum	1	1	1	1	1	1
78. Avicennia	1	1		1	1	1
79. Westringia	1					
80. Plantago	1	1		1	1	1
81. Boerhaavia	1			1	1	1
82. Pisonia	1	1	1	1	1	1
83. Rhagodia	1				1	1
84. Atriplex	1	1		1	1	1
85. Achyranthes	1			1	1	1
86. Muehlenbeckia	1	1				1
	82	49	31	65	66	71

	Australia.	New Zealand.	Norfolk Island.	Polynesia.	Malayan Archipelago.	Of wider range.
	82	49	31	65	66	71
87. Piper	1	1	1	1	1	1
88. Peperomia	1	1		1	1	1
89. Cryptocarya	1			1	1	1
90. Pimelea	1	1				
91. Exocarpus	1	1	1	1	1	
92. Viscum	1	1	1	1	1	1
93. Euphorbia	1	1	1	1	1	1
94. Hemicyclia	1				1	1
95. Baloghia	1		1	1		
96. Homalanthus	1			1	1	1
97. Celtis	1		1	1	1	1
98. Malaisia	1			1	1	
99. Ficus	1			1	1	1
100. Elatostema	1	1	1	1	1	1
101. Boehmeria			1	1	1	1
102. Parietaria	1	1		1	1	1
103. Dendrobium	1	1		1	1	1
104. Bulbophyllum	1	1		1	1	1
105. Cleisostoma	1				1	1
106. Microtis	1	1		1	1	1
107. Crinum	1		1	1	1	1
108. Moraea						1
109. Smilax	1			1	1	1
110. Geitonoplesium	1		1	1	1	1
111. Dianella	1	1	1	1	1	1
112. Commelina	1		1	1	1	1
113. Flagellaria	1			1	1	1
114. Juncus	1	1		1	1	1
115. Luzula	1	1		1	1	1
116. **Hedyscepe**						
117. Clinostigma				1		
118. **Howea**						
119. Pandanus	1		1	1	1	1
120. Cyperus	1	1	1	1	1	1
121. Scirpus	1	1	1	1	1	1
122. Gahnia	1	1		1	1	
123. Uncinia	1	1		1		1
124. Carex	1	1	1	1	1	1
125. Panicum	1	1	1	1	1	1
126. Oplismenus	1		1	1	1	1
127. Spinifex	1	1		1		1
128. Stipa	1	1			1	1
129. Sporobolus	1	1		1	1	1
130. Deyeuxia	1	1		1	1	1
131. Dichelachne	1	1	1			
132. Cynodon	1	1			1	1
	123	75	50	102	103	108

	Australia.	New Zealand.	Norfolk Island.	Polynesia.	Malayan Archipelago.	Of wider range.
	123	75	50	102	103	108
133. Chloris	1			1	1	1
134. Poa	1	1		1	1	1
135. Agropyrum	1	1		1	1	1
136. Lycopodium	1	1		1	1	1
137. Selaginella	1			1	1	1
138. Tmesipteris	1	1	1	1		
139. Psilotum	1	1	1	1	1	1
140. Cyathea	1	1	1	1	1	1
141. Hemitelia	1	1		1	1	1
142. Alsophila	1	1	1	1	1	1
143. Dicksonia	1	1		1	1	1
144. Hymenophyllum	1	1		1	1	1
145. Trichomanes	1	1	1	1	1	1
146. Davallia	1	1		1	1	1
147. Adiantum	1	1	1	1	1	1
148. Hypolepis	1	1		1	1	1
149. Cheilanthes	1	1		1	1	1
150. Pteris	1	1	1	1	1	1
151. Lomaria	1	1	1	1	1	1
152. Doodia	1	1	1	1	1	1
153. Asplenium	1	1	1	1	1	1
154. Aspidium	1	1	1	1	1	1
155. Polypodium	1	1	1	1	1	1
156. Notholaena	1	1	1	1	1	1
157. Platycerium	1				1	1
158. Todea	1	1		1		1
159. Marattia	1	1	1	1	1	1
	150	99	64	128	128	134

TABLE SHOWING THE DISTRIBUTION OF THE SPECIES INHABITING THE ISLAND.

	Australia.	New Zealand.	Norfolk Island.	Polynesia.	Malaya.	Of wider range.
1. Clematis glycinoides	1			1		
2. Drimys *Howeana*						
3. Stephania discolor	1			1	1	1
4. Lepidium foliosum	1					
5. „ ruderale	1					1
6. Hymenanthera latifolia			1			
7. Xylosma ovatum	1					
8. Pittosporum *erioloma*						
9. Calophyllum inophyllum	1			1	1	1
10. Hibiscus diversifolius	1		1	1	1	1
11. „ tiliaceus	1		1	1	1	1
12. Lagunaria Patersoni	1		1			
13. Oxalis corniculata	1	1		1	1	1
14. Pelargonium australe	1	1				1
15. Melicope *contermina*						
16. Evodia *polybotrya*						
17. Zanthoxylum Blackburnia			1			
18. Acronychia Baueri	1					
19. Dysoxylum Fraseranum	1					
20. Elaeodendron australe	1					
21. „ melanocarpum ...	1					
22. Cupania anacardioides	1					
23. Atalaya *coriacea*						
24. Dodonaea lanceolata	1					
25. Carmichaelia *exul*						
26. Mucuna gigantea	1			1	1	1
27. Canavalia obtusifolia	1			1		1
28. Vigna lutea	1			1		1
29. Sophora tetraptera		1				1
30. Caesalpinia Bonducella	1		1	1	1	1
31. *Colmeiroa carpodetoides*						
32. Leptospermum flavescens	1				1	1
33. Melaleuca ericifolia	1					
34. Acicalyptus *Fullagari*						
35. Metrosideros *nervulosa*						
36. „ polymorpha		1		1		
37. Passiflora Herbertiana	1					
38. Sicyos angulatus	1	1	1	1	1	1
39. Mesembryanthemum aequilaterale ...	1					1
40. „ australe ...	1	1	1			
	27	6	8	12	9	15

	Australia.	New Zealand.	Norfolk Island.	Polynesia.	Malaya.	Of wider range.
	27	6	8	12	9	15
41. Tetragonia expansa	1	1	1	1	1	1
42. ,, implexicoma	1	1				
43. Sesuvium Portulacastrum	1			1	1	1
44. Hydrocotyle hirta	1					
45. Apium prostratum	1	1	1	1		1
46. Panax *cissodendron*						
47. Randia *stipulosa*						
48. Psychotria *Carronis*						
49. Coprosma Baueri		1	1			
50. ,, *lanceolaris*						
51. ,, *putida*						
52. Brachycome *segmentosa*						
53. Olearia *Ballii*						
54. ,, *Mooneyi*						
55. Gnaphalium japonicum	1	1			1	1
56. ,, luteo-album	1	1	1	1	1	1
57. Cassinia *tenuifolia*						
58. Wedelia biflora	1			1	1	1
59. Bidens pilosa	1	1		1	1	1
60. Erechthites quadridentata	1	1				
61. Senecio *insularis*						
62. Scaevola Koenigii	1			1	1	1
63. Lobelia anceps	1	1				1
64. Wahlenbergia gracilis	1	1		1	1	1
65. Leucopogon Richei	1	1				
66. Dracophyllum *Fitzgeraldi*						
67. Myrsine crassifolia	1		1			
68. ,, *platystigma*						
69. Aegiceras majus	1			1	1	1
70. Sideroxylon *Howeanum*						
71. Symplocos Stawelli	1					
72. Jasminum didymum	1			1	1	1
73. ,, simplicifolium	1		1	1		1
74. Notelaea *quadristaminea*						
75. Olea paniculata	1			1		
76. Alyxia *Lindii*						
77. ,, ruscifolia	1			1		
78. ,, *squamulosa*						
79. Ochrosia elliptica	1			1		1
80. Lyonsia reticulata	1					
81. Vincetoxicum carnosum	1					
82. Tylophora *enervis*						
83. Marsdenia rostrata	1					
84. ,, *tubulosa*						
85. Geniostoma *petiolosum*						
86. Solanum aviculare	1	1	1			
	53	18	15	26	19	29

	Australia.	New Zealand.	Norfolk Island	Polynesia.	Malaya.	Of wider range.
	53	18	15	26	19	29
87 Solanum Bauerianum			1			
88. Ipomoea biloba	1			1	1	1
89. ,, bona-nox	1		1	1	1	1
90. ,, grandiflora	1			1	1	1
91. ,, palmata	1	1		1	1	1
92. Calystegia marginata	1	1	1			
93. ,, Soldanella	1	1	1	1		1
94. *Negria rhabdothamnoides*						
95. Tecoma austro-caledonica				1		
96. Eranthemum variabile	1			1		
97. Myoporum insulare	1					
98. Avicennia officinalis	1	1		1	1	1
99. Westringia rosmariniformis	1					
100. Plantago varia	1					
101. Boerhaavia diffusa	1			1	1	1
102. Pisonia Brunoniana	1	1	1	1	1	1
103. Rhagodia Billardierii	1					
104. Atriplex cinereum	1	1				
105. Achyranthes aspera	1			1	1	1
106. Muehlenbeckia axillaris	1	1				
107. Piper excelsum		1	1	1		
108. Peperomia reflexa	1		1	1	1	1
109. ,, Urvilleana		1	1			
110. Cryptocarya triplinervis	1					
111. Pimelea longifolia		1				
112. Exocarpus *homaloclada*						
113. Viscum articulatum	1			1	1	1
114. Euphorbia Sparmanni	1			1		
115. Hemicyclia australasica	1					
116. Baloghia lucida	1		1	1		
117. Homalanthus Leschenaultianus ...	1			1	1	1
118. Celtis *amblyophylla*						
119. Malaisia tortuosa	1			1	1	1
120. Ficus *columnaris*						
121. Elatostema reticulatum	1					
122. Boehmeria *calophleba*						
123. Parietaria debilis	1	1	1	1	1	1
124. Dendrobium gracilicaule	1					
125. ,, *Moorei*						
126. Bulbophyllum exiguum	1					
127. Cleisostoma *erectum*						
128. Microtis porrifolia	1	1		1		
129. Crinum pedunculatum	1			1	1	
130. Moraea *Robinsoniana*						
131. Smilax australis	1					
132. Geitonoplesium cymosum	1			1	1	1
	86	30	25	48	34	44

	Australia.	New Zealand.	Norfolk Island.	Polynesia.	Malaya.	Of wider range.
	86	30	25	48	34	44
133. Dianella caerulea	1					
134. Commelina cyanea	1		1	1		
135. Flagellaria indica	1			1	1	1
136. Juncus maritimus	1	1				1
137. Luzula *longiflora*						
138. *Hedyscepe Canterburyana*						
139. Clinostigma *Mooreanum*						
140. *Howea Belmoreana*						
141. „ *Forsteriana*						
142. Pandanus *Forsteri*						
143. „ (species imperfecte cognita)						
144. Cyperus haematodes...	1		1			
145. Cladium *insulare*						
146. Scirpus nodosus	1	1		1		1
147. Gahnia xanthocarpa		1				
148. Uncinia filiformis		1				
149. Carex breviculmis	1	1				1
150. „ gracilis	1					
151. Panicum sanguinale	1			1	1	1
152. Oplismenus compositus	1		1	1	1	1
153. Spinifex hirsutus	1	1		1		
154. Stipa micrantha	1	1				
155. Sporobolus indicus	1	1			1	1
156. Deyeuxia Forsteri	1	1				
157. Dichelachne crinita	1	1				
158. Cynodon Dactylon	1	1		1	1	1
159. Chloris Pumilio	1					
160. Poa caespitosa	1	1				
161. Agropyrum scabrum	1	1	1			
162. Lycopodium varium	1	1		1		1
163. Selaginella uliginosa	1					
164. Tmesipteris tannensis	1	1	1	1		
165. Psilotum triquetrum	1	1	1	1	1	1
166. Cyathea *brevipinna*						
167. „ *Macarthurii*						
168. Hemitelia *Moorei*						
169. Alsophila australis	1		1			
170. Dicksonia *nephrodioides*						
171. Hymenophyllum flabellatum ...	1	1				
172. „ minimum ...		1				
173. „ multifidum ...		1		1		
174. „ pumilum	1					
175. „ tunbridgense ...	1	1		1		1
176. Trichomanes apiifolium	1		1	1	1	
177. Davallia dubia	1					
178. Adiantum aethiopicum	1	1		1		1
	116	51	33	62	41	56

	Australia	New Zealand.	Norfolk Island.	Polynesia.	Malaya.	Of wider range.
	116	51	33	62	41	56
179. Adiantum hispidulum	1	1	1	1		1
180. Hypolepis tenuifolia	1	1		1	1	1
181. Cheilanthes tenuifolia	1	1		1	1	1
182. Pteris aquilina	1	1	1	1	1	1
183. ,, comans	1	1	1	1	1	1
184. ,, falcata	1	1			1	1
185. ,, incisa	1	1		1		1
186. ,, tremula	1	1	1	1		
187. Lomaria attenuata			1	1		1
188. ,, capensis	1	1		1	1	1
189. ,, *Fullagari*						
190. Doodia aspera	1					
191. Asplenium falcatum	1	1	1	1	1	1
192. ,, *melanochlamys*						
193. ,, Nidus	1		1	1	1	1
194. ,, obtusatum	1	1	1			1
195. ,, *pteridioides*						
196. Aspidium *apicale*						
197. ,, capense	1	1	1	1	1	1
198. ,, cordifolium	1	1		1	1	1
199. ,, molle	1	1		1	1	1
200. Polypodium australe	1	1				1
201. ,, confluens	1		1	1		
202. ,, Hookeri	1			1		1
203. ,, punctatum	1	1				1
204. ,, pustulatum	1	1				
205. ,, tenellum	1	1	1	1		
206. Notholaena distans	1	1	1	1		
207. Platycerium alcicorne	1					1
208. Todea *Moorei*						
209. Marattia fraxinea	1	1		1	1	1
	141	71	45	81	53	76

The foregoing enumeration, and the tables of distribution of the genera and species of vascular plants hitherto collected in Lord Howe Island, offer ample material for discussion and speculation. With regard to the enumeration, in the opinion of Mr. C. Moore, who has himself visited and botanized the island, it is not exhaustive, as may be gathered from the fact that he has long deferred an intention to write a Flora of the island, because no opportunity has occurred for further botanical exploration. Still it is not probable

that any plants remain undiscovered that would materially modify the deductions concerning the origin and affinities of the flora to be drawn from those already known.

With regard to the tables showing the distribution of the genera and species, it should be borne in mind that they are only approximations, subject to modification according to divergencies of opinion on the limitation of genera and species. But a glance at the list is sufficient to convince one that the genera, with exceedingly few exceptions, are well known and generally accepted; and a personal knowledge of most of the species inclines me to the belief that the number given as evidence is susceptible of reduction rather than augmentation. Another source of slight error may result from incomplete data. For instance, I think it is highly probable that several species not indicated as occurring in Norfolk Island really exist there.

I will now proceed to an analysis of the constituents of the flora of the island, to be followed by some remarks on the absence of certain groups characteristic of the nearest insular and continental areas. First with regard to numbers. There are 209 species, belonging to 160 genera, and 70 natural orders, grouped as follows:—

	Orders.	Genera.	Species.
Dicotyledons	55	102	123
Monocotyledons	12	33	38
Vascular Cryptogams	3	25	48
Totals	70	160	209

There are four endemic genera, namely, *Colmeiroa* (Saxifragaceae), *Negria* (Gesneraceae), and *Hedyscepe* and *Howea* (Palmae); and there are fifty endemic species, indicated in the table by *italics*. Of these thirty-three are dicotyledons; eight monocotyledons, and nine vascular cryptogams. These statistics reveal the phenomena characteristic of remote insular floras; that is to say, a relatively large number of orders and genera in proportion to the number of species; a preponderance of shrubs and trees over herbaceous plants;

a small number of monocotyledons, especially of the petaloid group, and a considerable endemic element. Among the insular floras that I have worked out, this is most nearly comparable to that of Juan Fernandez; both islands being situated in nearly the same latitude, several hundred miles distant from the nearest land, and rising approximately some 3,000 feet above the sea-level. But the two floras present some singular divergencies in details. Juan Fernandez is more than twice the size of Howe; yet its vascular plants number only 162 species, as against 209, and of these no fewer than forty-four are ferns. On the other hand, the endemic element in Juan Fernandez is about fifty per cent., or double that of Howe, and arboreous Compositae characteristic of the former, of St. Helena and the Galapagos, are wanting in the latter. They have, however, many features in common, and there are even remote affinities between the two floras. Thus the essentially southern genera *Drimys*, *Coprosma*, and *Uncinia* are represented by different species in the two islands. The only leguminous plant in Juan Fernandez, *Sophora tetraptera*, is also found in Howe. *Lobelia anceps* is another plant common to the two islands, but little stress can be laid on this fact, as it probably owes its present wide range to human agency. The Tree-ferns of Juan Fernandez belong to the genera *Dicksonia* and *Alsophila*, both represented in Howe, and the solitary endemic palm, *Juania australis*, belongs to the Areceae, and is placed very near the genus *Howea*. The presence of endemic genera of arboreous Compositae in Juan Fernandez, each represented by several species, is the principal distinguishing feature. Yet arboreous Compositae are not wanting in the New Zealand region, *Eurybia Traversii* and *Senecio Huntii* being conspicuous trees in the woods of the Chatham Islands, to say nothing of those inhabiting New Zealand itself.

I will now examine a little more in detail the table showing the distribution of the genera of the vascular plants of Howe Island. As we have seen, out of a total of 160, there are only four endemic. This is a very low percentage,

considering that thirty per cent. of the Australian, and about 6·5 per cent. of the New Zealand genera are endemic. Ten of the forty-six genera of flowering plants in Juan Fernandez are endemic. In St. Helena the numbers are, total, twenty-six and five endemic; and in the more extensive Hawaiian flora fifteen per cent. of the genera are endemic. I may add that the Australian and New Zealand outlying islands generally have very few endemic genera; whereas New Caledonia's remarkably rich flora abounds in endemic genera. Of the Howe Island genera 151, out of 160, are Australian; ninety-nine are represented in New Zealand and sixty-four in Norfolk Island – the last number probably too low. But the most remarkable fact is that no fewer than 134 of the genera reach the sixth column of the table, which means that they extend in some direction, or directions, beyond the Australasian, Polynesian, and Malay Archipelago regions—these regions taken in their widest sense. Some of the genera, indeed many of them, are of world-wide range; but I do not intend following up their full distribution. What is more remarkable, is the smallness of the number of purely Australasian genera, which do not exceed a dozen; they are: *Hymenanthera*, a shrubby genus of Violaceae; *Lagunaria* (Malvaceae); *Melicope* (Rutaceae), but this also extends to Polynesia; *Carmichaelia* (Leguminosae), otherwise restricted to New Zealand; *Acicalyptus* (Myrtaceae), New Caledonia and Fiji; *Cassinia Brachycome*, and *Olearia* (Compositae); *Notelaea* (Oleaceae); *Lyonsia* (Apocynaceae); *Westringia* (Labiatae) and *Dichelachne* (Gramineae). It will be noticed that the specially characteristic Australasian genera are not represented in Howe Island.

Respecting other genera, I may mention that the supposed African *Myoporum* has been described as a new genus (*Zombiana*) by Baillon, and the Madagascar plant referred to *Exocarpus* turns out to be Leguminosae,—*Phylloxylon*, Baill., *Neobaronia*, Baker. *Ficus* is not represented in New Zealand, and, so far as is known, not in Norfolk Island. It is also worthy of note that the world-wide genus *Juncus*

has not been found in the whole of Polynesia, if we except New Caledonia; but *Luzula* reaches the Sandwich Islands. Passing to the table showing the distribution of the Howe Island species, the total number, as already pointed out, is large for a remote island; yet the endemic percentage is low, both as compared with other less isolated islands and continental areas of greater extent. As compared with a similar area, say, in the South of England, the number of *species* is decidedly small; but it should be remembered that the increase in the number of species is in the inverse ratio to the increase of area. Thus a single county of England contains something approaching two-thirds of the species found in the whole of the counties.

The most interesting point in the distribution of the non-endemic species of Howe Island is their extensions beyond the island. Leaving out the sporiferous plants, 55 out of 160 have a wide distribution; including the sporiferous plants, 79 out of 209; and this number includes only a small proportion of the plants known to be dispersed by oceanic currents and birds. Taking those species which do not extend beyond the countries indicated in the first three columns, namely, Australia, New Zealand, and Norfolk Island, we find that thirty-one are found elsewhere only in Australia; five only in New Zealand; three only in Norfolk Island; twelve only in Australia and New Zealand combined; four only in Australia and Norfolk Island; one only in New Zealand and Norfolk Island; and four extend to Australia, New Zealand, and Norfolk Island, but are not found elsewhere. Adding these fifty-eight to the fifty endemic species, we have something more than half of the total coming under the head of what I should term Australasian species. The proportion of Australasian species would be slightly augmented by leaving the Ferns out of the calculation, as Ferns generally have a wider range than flowering plants. The total number of known extensions[1] to Australia is 142; to New Zealand,

[1] I use the word 'extension' here in a conventional sense, of course; not in the sense of the island being the centre from which the plants have actually spread.

72; to Norfolk Island, 39; to Polynesia, 82; to the Malay Archipelago, 54; and the extensions beyond the countries named, 79.

Now a few facts with regard to the absence of certain elements from the flora. It has already been mentioned that few of the plants known from actual observation to be dispersed by ocean currents and birds are found in the island. All the Leguminosae in the island, however, with the exception, perhaps, of the *Carmichaelia*, belong to this category, as well as several species of *Ipomoea*. The characteristic shrubs and trees of tropical and subtropical Polynesia, such as *Thespesia*, *Pemphis*, *Barringtonia*, *Suriana*, *Guettarda*, *Morinda*, *Cordia*, and *Tournefortia* are wholly absent. Gymnosperms and Proteaceae are unrepresented, though both are present in New Zealand and Norfolk Island. The commonest Australian types, abundant in Tasmania, such as *Eucalyptus*, *Acacia*, and *Casuarina*, are wanting here, as they are also in New Zealand. The liliaceous genus *Cordyline*, a conspicuous feature in New Zealand, Tasmania, Australia, and Norfolk Island, though not restricted to these countries, might have been expected to occur. Equally singular is the absence of *Ranunculus*, *Epilobium*, and *Veronica*, genera of world-wide distribution, so copiously developed in New Zealand, where they are exceedingly numerous in species, and not uncommon in Australia, alike in lower latitudes and lower altitudes. Poverty in Leguminosae it shares in common with New Zealand, Norfolk Island, and the distant Juan Fernandez and St. Helena, to say nothing of the numerous smaller southern islands. I might continue these comparisons, but enough has been said to give an idea of the composition of this peculiar small flora, the affinities of which are somewhat complex. The endemic element in this, as in so many other insular floras, has been much over-estimated by some writers, and this has often led to false or improbable deductions. After eliminating all plants likely to have been introduced, accidentally or intentionally, the specific endemic element does not exceed 25 per cent., whereas in New

it ranges from 60 to 85 per cent. Yet it has been objected that a former land-connexion between New Zealand and Howe Island was improbable on account of the endemic character of the flora of the latter. This is the view taken by Drude. Tchihatchef states that the flora has little affinity with that of Australia, and belongs to the same centre as Norfolk Island. Wallace, Engler, and others favour a considerable former land-connexion in this region; and looking at the statistics, and the mixed character of the flora, when considered in connexion with that of New Zealand, Norfolk Island, and East Australia, this seems the only sound explanation. The figures given, pp. 272–281, make it difficult to decide where the strongest affinities lie, though when we consider the size of the different areas there is little to choose between them. The number of species common to the island and Australia only, is far in excess of those common to the island and to New Zealand and Norfolk Island combined. The next highest in these comparisons is the number common to the island and to Australia and New Zealand combined. Still we cannot determine the affinities by mere numbers.

As to the flora being derived rather than the remains of a former more extensive one, I think the evidence is all in favour of the latter view, or we must suppose a former much more extensive interchange of plants than under existing agencies could possibly take place. For many years I have been collecting evidence bearing on the dispersal of plants, and I think that all the conveying agencies combined are insufficient to account for the present flora of Lord Howe Island.

In conclusion, I may repeat that the prominent features in the vegetation of Howe Island are the Palms, Screw-Pines, Tree-Ferns, and Banyans; and specially noteworthy among the rare endemic plants are *Moraea Robinsoniana* and *Dracophyllum Fitzgeraldi*. Excepting the Seychelles, where there is an even greater development of Palms, no other remote

island is remarkable for its palm-vegetation, apart from the Coco-nut Palm, which is wanting here, as well as in Norfolk Island, where, as well as in New Zealand, there is one endemic species of this family. The extreme rarity of Palms, except the Coco-nut, in Eastern Polynesia, may, however, be due to the hand of man, as the existence of two or three very rare endemic forms suggests.

WORKS CITED.

BECCARI, O.—Malesia, i, p. 66. 1877.

BENTHAM, G.—Flora Australiensis, 7 vols., 8vo. 1863–78.

DRAKE DEL CASTILLO, E.—Flore de la Polynésie Française, 1 vol., 8vo, pp. 352. 1893.

DRUDE, O.—Geographisches Jahrbuch, xv, p. 396. 1892.

ENDLICHER, S. L.—Prodromus Florae Norfolkicae, 1 vol., 8vo, pp. 100. 1833.

ETHERIDGE.—Lord Howe Island; its Zoology, Geology, and Physical Characters, 1 vol., 8vo, pp. viii and 132, plates 10. 1889.

HEMSLEY, W. B.—Report on the Botany of the Voyage of H. M. S. *Challenger*, 1 vol., 3 parts. 1884–85.

———The Flora of the Tonga or Friendly Islands, in the Journal of Linnean Society, xxx, pp. 158–217, plates 9–11. 1894.

HEWARD, R.—Biographical Sketch of the late Allan Cunningham, Esq., in Hooker's London Journal of Botany, i, pp. 107–128. 1842.

———List of Plants detected on Norfolk Island by Mr. Cunningham that are not enumerated in Endlicher's Prodromus Florae Norfolkicae, in note on pp. 121–124.

HILLEBRAND, W.—Flora of the Hawaiian Islands, 1 vol., 8vo, pp. 673. 1888.

HOOKER, J. D.—Handbook of the New Zealand Flora, 1 vol., 8vo, pp. 798. 1864.

HUNTER, J.—An Historical Journal of the Transactions at Port Jackson and Norfolk Island, 1 vol., 8vo, pp. 525. 1793.

MAC GILLIVRAY, J.—Letters from, Naturalist on H. M. S. *Herald*, in Hooker's Kew Journal of Botany, vi. pp. 353–363. 1854.

MOORE, C.—Sketch of the Vegetation of Lord Howe's Island, 4to, pp. 4. 1869.

M[OORE], C.—Lord Howe's Island, in Gardeners' Chronicle, 1869, p. 968.

MOORE, C.—Remarks on the Botany of Lord Howe's Island in Transactions of the Royal Society of New South Wales, v, pp. 29–34. 1872.

MUELLER, F. VON.—Fragmenta Phytographiae Australis, vols. vii–ix. 1869–1875.

MUELLER, F. VON.—Index omnium Insulae Howeanae Plantarum, quas hactenus obtenui, exclusis speciebus certe introductis, in Fragmenta Phytographiae Australis, ix, pp. 76–78. 1875.

MUELLER, F. VON.—Second Systematic Census of Australian Plants, 1 vol., 4to, pp. 244. 1889.

PHILLIP, A.—The Voyage of, to Botany Bay, 1 vol., 4to, pp. 298. 1789.

SEEMANN, B.—Flora Vitiensis, 1 vol., 4to. 1865–73.

TATE, R.—On the Affinities of the Floras of Lord Howe and Norfolk Islands, in Report of the Fourth Meeting of Australasian Association for Advancement of Science, p. 407. 1892. (Title only published.)

TATE, R.—On the Floras of Norfolk and Lord Howe Islands, in the Macleay Memorial Volume of Linnean Society of New South Wales, pp. 205-221. 1893.

TCHIHATCHEF, P. DE.—Végétation du Globe, ii, p. 797. 1878.

VOLLMER, A.—Lord Howe Insel, Pitcairn und Norfolk, in Petermann's Mittheilungen, xli, pp. 72–77. 1895.

WENDLAND, H., and DRUDE, O.—Palmae Australasicae, in Linnaea, xxxix, pp. 153–238. 1875.

WHITE, J.—Journal of a Voyage to New South Wales, 1 vol., 4to, p. 299. 1790.

WILSON, J. B.—Report on the Present State and Future Prospects of Lord Howe Island, 1 vol., 4to. With photographs. 1882.

Geology by H. WILKINSON ; Botany by J. DUFF.

SUPPLEMENTARY NOTE.—After the manuscript of the foregoing paper had passed into the printer's hands, the Macleay Memorial Volume of the Linnean Society of New South Wales came under my notice. This contains a paper by Professor R. Tate on the same subject, the title of which is given above.

Professor Tate's work has enabled me to make a few corrections, and two or three additions. His distribution-tables differ from mine in many small and unimportant details, in part doubtless owing to the difficulties of synonymy and the publication of incorrect names for some of the plants. I have not thought it desirable to modify or add to my distribution-tables from this source, because I think that in some cases, at least, the distribution given is doubtful, whilst in others it is incomplete. Thus the following Howe plants are recorded from Norfolk Island, though they do not appear in my table:—*Drimys Howeana*, *Metrosideros polymorpha*, *Viscum articulatum*, *Euphorbia Sparmanni*, *Malaisia tortuosa*, *Microtis porrifolia*, *Geitonoplesium cymosum*, *Sporobolus indicus*, and *Hymenophyllum multifidum*. There is no great improbability about any of these, and I have found specimens of the *Viscum* and *Geitonoplesium*; but the absence of *Drimys* and *Metrosideros* from our collections makes me a little doubtful concerning these two. He further records *Lepidium foliosum* and *Mesembryanthemum aequilaterale* from New Zealand, but there are no specimens at Kew. *Echinopogon ovatus* (Gramineae) and *Aspidium decompositum*, both common New Zealand and Australian plants, are additions to my list.

I have also been able to add the title of another paper by Mr. C. Moore, previously overlooked by me, and not seen by Professor Tate. In this he deals with the distribution of the genera only of Howe Island plants, and arrives at the conclusion that this island, Norfolk Island, New Zealand, New Caledonia, and some parts of Australia were formerly connected. He mentions the genera *Premna* and *Pipturus*. The specimen referred to the former is flowerless and indeterminable; and the latter is probably a slip, and should be *Elatostema*.—W. B. H.

NOTES.

RESPIRATION AND ASSIMILATION IN CELLS CONTAINING CHLOROPHYLL.—It is a matter of common knowledge that free oxygen is essential to the continuance of active movement on the part of the protoplasm of the higher plants. An apparent—but only apparent—contradiction to this generalization is, however, furnished by the behaviour of the rotating protoplasm of an *Elodea* leaf when surrounded by an atmosphere of hydrogen gas. The experiment about to be described is a well-known one, but the conclusions which can be drawn from it would appear not to be so generally appreciated as they seem to deserve.

If a healthy leaf of *Elodea* be placed in one of those extremely convenient tubes designed by Professor Marshall Ward[1], the movement of the protoplasm can be studied under favourable circumstances. Indeed it is possible to keep the same leaf, with its moving protoplasm, under observation for several days. If a current of hydrogen be passed through such an apparatus containing a vigorous *Elodea* leaf, and the precaution be taken to dip the end of the tube through which the gas is escaping under mercury or water (to prevent the possibility of diffusion of air), it is clear that in a short time the pressure of oxygen within the chamber must speedily be reduced to what is practically zero. But if the experiment be conducted in the day-time, it is found that no arrest of the protoplasmic movement takes place. Indeed, in an experiment (which has been several times repeated in my laboratory), exposure to hydrogen from 10 a.m. to 5 p.m. produced no effect whatever on the rapidity of the rotation within the plant-cells.

[1] See Phil. Trans. Vol. clxxxiii (1892), B, pp. 131, 132, where a description and figures of the apparatus here referred to are given.

But it is obvious that since the protoplasm was thus manifesting active vitality, respiration must have been also going on, and the only possible explanation of the process seemed to be that as fast as the carbon dioxide was produced, as the result of respiration, it was split up and the oxygen liberated by the chlorophyll-granules under the influence of the daylight. The oxygen thus would be again rendered available for purposes of continued respiration. Blackman's[1] researches have shown that it is highly probable that during active assimilation, no carbon dioxide passes out from the leaves, but that it is decomposed and utilized as fast as it is produced. The observation just recorded seems to confirm his conclusions on this point in a striking manner. Of course it is quite possible that a certain small amount of the gases may pass out along with the hydrogen-stream, but enough oxygen remains to allow of respiration being maintained. Clark's investigations[2] have, moreover, shown that a very low pressure of oxygen (about 1·3 mm. of mercury) is sufficient to enable protoplasmic movement to go on.

If the apparatus be covered over by a dark box, so as to exclude the light whilst the experiment is still proceeding, the movement of the protoplasm entirely ceases in a time varying in different experiments from two to five minutes, but it recommences on the light being once more admitted. This interference—the result of withdrawal of light—can be repeated many times, and recovery takes place as often afterwards. This seems to furnish a strong argument in support of the explanation of the phenomena here put forward, for it can be easily proved (in a control-experiment) that the mere exclusion of light from the leaf, the protoplasm of which is in a state of movement, produces no obvious effect so long as ordinary air is circulating through the apparatus.

Again, in the case of hairs which do not contain chlorophyll, the presence or absence of light makes no difference as regards their behaviour when exposed to an atmosphere of hydrogen. All movement is arrested, usually in a period varying from two to five minutes. I may add that the hairs present in the throat of the corolla of the White Dead-Nettle (*Lamium album*) are very suitable objects on which

[1] Experimental Researches on Vegetable Assimilation and Respiration, Phil. Trans. Vol. clxxxvi (1895), B.

[2] James Clark, Ueb. d. Einfluss niederer Sauerstoffpressungen auf d. Bewegung d. Protoplasmas. Ber. d. deutschen bot. Gesellsch., vi (1888).

to experiment on protoplasmic movement. They are easily prepared by cutting rather thick transverse sections of the corolla, and mounting in water. They react quickly to various stimuli, and they possess the additional advantage of being readily accessible during the greater part of the year.

I also tried the effect of exposure to an atmosphere of hydrogen on *Ophrydium versatile*, a colonial protozoan consisting of a large number of green Vorticella-like animals imbedded in a common gelatinous matrix. An exposure to hydrogen for half an hour in bright daylight produced no apparent effect, but after continuing the treatment for a further quarter of an hour, the large cilia had become sluggish in their movement. The experiment was then discontinued. In another series, a group of *Ophrydia* which had been for twenty minutes in an atmosphere of hydrogen, but seemed to be still perfectly healthy, were covered over with a dark box. After the light had been excluded for three minutes, a number of the organisms were found to have been killed, and a further exposure to the gas in the dark for three minutes resulted in the death of them all. They contracted, and quite suddenly burst, the green matter streaming out as globules into the water from the glairy mass of the disintegrating body of the animal.

This experiment shows that the utilization of some oxygen-containing substance (probably the carbon dioxide evolved during respiration) takes place under suitable conditions of illumination, and it may probably be concluded that the evolution of oxygen available for the continuance of the respiratory process is connected with the presence of the green colouring-matter already referred to. But it would seem that the balance between the two processes of assimilation and respiration, between the evolution and utilization of oxygen, is not so accurately maintained in this instance as in that of *Elodea*; since after three quarters of an hour's exposure to the atmosphere of hydrogen, the activity of the animal was clearly waning, although the conditions of illumination appeared to be in all respects favourable.

In 1887 Pringsheim published a series of researches on the behaviour of *Chara* in an atmosphere of hydrogen to which a small amount of carbon dioxide had been added[1]. He states that the

[1] Pringsheim, Ueb. d. Abhängigkeit der Assimilation grüner Zellen von ihrer Sauerstoffathmung, &c. Sitzungsber. d. k. Preuss. Akad. d. Wiss. z. Berlin,

moving protoplasm is brought to rest in a period varying from two to twelve hours, and that it seemed to make no difference whether light were excluded or not. I repeated the experiment on *Nitella*, but used pure hydrogen instead of the mixture of gases employed by Professor Pringsheim. Possibly the discrepancies existing between our observations may be partly due to this fact. *Nitella* and also *Chara* are somewhat difficult plants to work with; and often, for no apparent reason, fail to give conclusive results unless one is very careful to select thoroughly healthy cells in which the rotation is very active. I find that under these circumstances one can keep the cell in an active condition, in an atmosphere of hydrogen, for a whole day when exposed to light. If light be at any time excluded, by means of the darkened box already mentioned, the movement becomes slow in about twenty minutes, and in half an hour usually, though not always, ceases. On re-exposure to light, rotation rapidly begins again, and in most cases completely recovers its old rate in about one or two minutes. If the cell be now darkened once more, a slowing down, or even complete arrest, of the protoplasmic rotation is effected in *five to seven minutes*. This experiment I have repeated a large number of times, and it may be safely shown as a class-demonstration. A longer re-exposure to light necessitates a more prolonged continuance of darkness in order to reproduce the quiescent condition.

The experiments with *Nitella* were further checked by similar ones, carried on simultaneously with *Elodea*, as detailed above, and the differences are, I think, susceptible of an easy explanation.

The cell of *Nitella* is a very large one, and consequently the volume of its cell-sap is *relatively* (as well as absolutely) greater than that in *Elodea* as compared with the amount of protoplasmic substance. Hence, supposing the cell-contents to be saturated to an equal extent with oxygen, it is clear that *Nitella* would be in a more favourable position when the further supply of oxygen was cut off than is *Elodea*. We might expect then, assuming the cells to be similar in other respects, that the movement of the protoplasm in *Nitella* would continue after that in the other plant had come to rest, and this is found to be the case. But, on the whole, the experiments seem also to show that *Nitella* can do with *less* oxygen than *Elodea*, for the difference in relative bulk in the two cases is hardly sufficient to

xxxviii. 1887. A critical abstract of this paper is given by Professor Vines in Annals of Botany, Vol. i, p. 371 et seq.

account for all the diversity in their respective reactions. These results and considerations support Professor Vines' criticisms passed on Pringsheim's conclusions as to the seat of origin of the oxygen evolved during assimilation. Further discussion of the many points here raised would, however, be out of place within the limits of a note, but I hope to return to the subject at length on a future occasion.

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A NEW CASE OF POLYSTELY IN DICOTYLEDONS.—The stems of Angiosperms and the roots of vascular plants in general may be considered as exhibiting almost universally a monostelic structure. As regards the former, the only exceptions hitherto described are the Gunneras, and the Primulas of the section *Auricula*, which are polystelic, and the Nymphaeaceae with a few others, chiefly water-plants, which are astelic. In the case of roots, no exception was known until polystely was recently discovered in the adventitious roots of certain Palms, *Areca*, *Verschaffeltia*, &c., by Cormack[1].

While making a comparative examination of certain Nymphaeaceae, the results of which I hope to publish fully at a later time, I have found that in addition to astely, this remarkable order also presents cases of polystely.

In certain tropical and sub-tropical species of *Nymphaea*, the plants, at the end of each vegetative period, form numerous tubers as a means of surviving through the approaching dry season. Some, as *Nymphaea rubra*, *N. stellata*, &c., according to Raciborski[2], convert the whole of the lower part of the rhizome into a tuberous starch-laden structure coated with periderm. Others, as *N. flava* and *N. tuberosa*, examined by me, bear their tubers on lateral stalks of greater or less length. In *N. flava* they form very long stolons or runners (30–40 c. m. long), which are but slightly thickened at their terminations, where they bear below a large number of swollen starch-laden sausage-shaped roots, and above a number of buds protected by scale-leaves. These runners exhibit a truly polystelic arrangement of their vascular tissues, four to five groups of which are found running in the lacunar ground-tissue, each consisting of three to four vascular bundles grouped around a common centre where their xylems are confluent.

[1] In a paper read before the Linnean Society early this year.

[2] Raciborski, Flora, Band lxxviii, p. 262, 1894.

The well-formed and prominent phloem-groups are, on the other hand, completely separate from one another. The centre of each group, which thus constitutes a distinct stele, is occupied by an intercellular space formed by the disintegration of the older elements of the xylem, which, however, are still persistent towards each end of the runner. Around each stele runs a well-developed and easily demonstrated endodermis.

In *Nymphaea tuberosa* the stalks which bear the tubers are very short, not more than 4 c.m. long, some of the tubers being almost sessile. But all cases examined showed essentially the same structure as in *N. flava*, the stalk containing three to five separate steles, each composed of three to five vascular bundles, although here the separation of the bundles within each stele is much less distinct.

In all cases, whether the base only of the rhizome becomes tuberous (Raciborski, l. c.), or whether the tubers are borne on shorter or longer stalks, and even when the tubers are metamorphosed flower-buds, as in *Nymphaea Lotus var. monstrosa* [1], at the commencement of the next period of vegetation they bear buds, the first or first two internodes of which grow out into thin stolons and at their terminations swell out to form new rhizomes. In the cases of *N. flava* and *blanda*, these stolons, which may be termed secondary, arise from the buds previously mentioned in reference to the former species, as springing from the ends of the primary runners. They possess six to seven vascular bundles, the arrangement of which differs from one stolon to another, and even in the different regions of the same stolon. For they may be all separate and distinct, or a varying number of them may unite in pairs. When there are only six present, and these unite into three pairs, such sections present a striking similarity to those of the floral peduncle of *Cabomba aquatica*, for here also there are six bundles of a very similar appearance and united into three pairs. The rhizome and floating shoots of *Cabomba* possess two such pairs only.

These facts seem to introduce a question as to whether the term 'stele' should be applied to these paired bundles or not, for in the former case we should have to consider *Cabomba*, and also *Brasenia*, which is exactly like it, as polystelic plants throughout, excepting their petioles. At any rate, we have here in the single order Nymphaeaceae all gradations from the undoubted distinct steles found in the primary

[1] Barber, Ann. Bot., Vol. iv, p. 105.

stolon of *Nymphaea flava*, which contain five, four, or as few as three bundles each, to those with two bundles in the secondary stolons of the same plant and of *N. blanda*, and also in the stem and floral peduncles of *Cabomba*. Indeed, in the secondary stolons of *N. flava* and *N. blanda* the two bundles of any pair may in their course fuse completely together and become a single bundle comparable to a bundle in the stem of *Nelumbium nuciferum*.

We see then that in this order a complete series of transitions can be traced from typical polystely to the well-known astelic condition.

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DEMONSTRATION OF ROOT-PRESSURE AND TRANSPIRATION.—I have found that root-pressure may be conveniently demonstrated and measured by means of an ordinary Bourdon's pressure-gauge. To give an instance:—a few weeks ago, one of these instruments, connected with a branch of a Vine growing in the open, gave readings up to a maximum pressure of 10 lbs. to the square inch. I am arranging to have an instrument of this kind made with a special view to this purpose, provided with an adaptation for obtaining a graphic tracing on a revolving drum, and I am inclined to think that it may be possible by this means to obtain more accurate knowledge than we at present possess on such points as the daily period of the root-pressure and the influence upon it of variations in the external conditions.

With regard, now, to transpiration, the recent researches of Dixon and Joly[1], and of Askenasy[2], seem to make the determination of the suction-force of a transpiring plant a point of great importance. Boehm[3] succeeded in causing a transpiring branch to raise a column of mercury 900 m.m.—that is, the branch exerted a suction-force of about 140 m.m.—and that is all the information that we possess at present in the way of measurement of the force which is now regarded as the efficient cause of the transpiration-current.

A year ago I attempted to make some measurements of this force

[1] Dixon and Joly: On the Ascent of Sap; Annals of Botany, Vol. viii, 1894; Proc. Roy. Soc., Vol. lvii, 1895; Phil. Trans. 1895.

[2] Askenasy: Ueber das Saftsteigen; Verhandl. d. Naturhist.-med. Vereins zu Heidelberg, Neue Folge, Bd. v, 1895 and 1896.

[3] Boehm: Ber. d. deut. bot. Ges. 1893.

by placing branches in air-tight connexion with a closed vessel of water communicating with a mercurial barometer. My experiments were made with not very large branches, and were few in number as they had to be suddenly brought to a close; but some of the results were sufficiently striking. Thus, with a branch of Cherry-laurel, the mercury sank 190 m.m. in four hours; that is, the suction-force of the branch was equal to one-fourth of an atmosphere.

I am now resuming the experiments, substituting a Bourdon's[1] vacuum-gauge for the mercurial barometer, and with still more striking results. Thus, a vigorous branch of Beech, bearing about 1,100 leaves, gave an indicated suction-force of rather over twenty-one inches of mercury (about 530 m.m.) within one hour. As I did not succeed in obtaining a higher reading, I am inclined to regard this as a measure of the suction-force of this branch. The record is briefly as follows:—

2·40 p.m.	indicator of gauge	=	0	inches of	mercury
2·50 „	„ „	=	14	„	„
3·00 „	„ „	=	18½	„	„
3·25 „	„ „	=	21	„	„
3·40 „	„ „	=	21	„	„

Some indication of the nature of the relation between the suction-force and the leaf-area of a branch is afforded by the remarkable fact that the gradual reduction, to the extent of 900, of the number of leaves borne by the above-mentioned branch of Beech, produced no proportionate — or even perceptible — diminution in the indicated suction-force. Full details will appear in a subsequent number of the Annals.

S. H. VINES.

THE DIGESTIVE FERMENT OF NEPENTHES.—In view of statements which have appeared of late years throwing doubt upon the digestive function of the pitchers of *Nepenthes*, and upon the possibility of obtaining from them a peptic enzyme, I have thought it worth while to repeat the experiments on the subject which I made in the year 1876. I propose to publish a full account of these repeated experiments in a future number of this periodical: but I think it well to take this early opportunity of stating that, so far as the work has been carried at present, the results completely accord with those at which I arrived twenty years ago.

S. H. VINES.

[1] The Bourdon's gauges used in these experiments were kindly lent to me by Messrs. Elliott Bros. of St. Martin's Lane, London.

CHEMISTRY OF LICHENIC AND FUNGAL MEMBRANES[1].

In this investigation the Lichens *Cetraria islandica*, *Peltigera canina* and *Evernia prunastri*, and the sclerotium of *Claviceps purpurea*, were subjected to examination. The work was suggested by a research of Winterstein[2], who has found chitin or a similar compound in the membranes of several Fungi. It was expected to find that the hyphal membranes of the Lichens would contain this substance, and the algal ones cellulose, as essential constituents. The sclerotium of *Claviceps* was examined to prove, if possible, the identity of mycosine[3] with chitosan[4].

I was unable to prove the presence of chitin or a similar body in *Cetraria*. The hyphal membranes appeared to consist principally of lichenin, and of a paragalactan or a mixture of several. Lichenin seemed to be a galactan, yielding an osazone melting at 191°–192° C. The algal membranes consisted principally of a cellulose, probably gluco-cellulose. From the hyphal membranes of *Peltigera*, a substance with physical properties agreeing tolerably with those of chitosan was obtained. The yield was small, and analysis did not give percentages reconcilable with those of chitosan. The algal membranes did not consist of cellulose. Lichenin was proved to be absent. No substance comparable with chitosan appeared to be with certainty obtainable from the hyphae of *Evernia*. The principal constituent seemed to be a substance which swelled greatly on treatment with hydrochloric acid and a solution of potassium-hydroxide, and disappeared during fusion with the latter. This compound was not examined. The algal membranes consisted of cellulose, probably gluco-cellulose. No cellulose could be obtained from the hyphal membranes of any one of these Lichens.

The sclerotium of *Claviceps* yielded a substance for the most part similar in physical properties to chitosan. Analysis did not confirm its identity with this substance, and the percentages differed from those of mycosine. A large fraction of the hyphal membranes seemed to consist of a compound, or several, which yielded during fusion one or more aliphatic acids; these were not examined for want of time.

[1] Abstract of a paper which will appear in Hoppe-Seyler's Zeitsch. für phys. Chem.

[2] Hoppe-Seyler's Zeitsch. für phys. Chem., Bd. xx, 1894; Bd. xxi, 1895.

[3] E. Gilson: 'Recherches chimiques sur la membrane cellulaire des champignons.' Extrait de la Revue 'La cellule,' t. xi, 1 fascicule.

[4] Hoppe-Seyler: Ber. d. deutsch. chem. Ges., 1894, S. 3329.
T. Araki: Hoppe-Seyler's Zeitsch. für phys. Chem., Bd. xx, S. 498 et seq.

For comparison I give the percentages obtained by analysis of the substances from *Peltigera* and *Claviceps*, as well as those given for mycosine and chitosan.

	Mycosine	Chitosan	*Peltigera*	*Claviceps*	
				I	II
C.	43·74%	43·97%	41·69%	41·33%	41·33%
H.	7·30„	6·80„	5·00„	6·79„	6·10„
N.	7·31„	7·32„	1·35„		

Nitrogen was present in the substance from *Claviceps*, but in such small amount that I do not think it could have approached anything like 7·32%. I had no time to conduct an analysis.

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DIAHELIOTROPISM OF RADIAL MEMBERS. — The purpose of the present note is to call attention to the fact that the radial stems of *Pellionia Daveauana* afford a very good example of diaheliotropic irritability in a radial member. The stems of this plant normally grow parallel to the surface of the ground, the leaves lying in the horizontal plane with their superior surfaces uppermost. If the plants are suspended freely, and exposed to bright diffuse daylight falling parallel to the length of the stem, when the apex is directed towards the source of light the stem curves downwards; and when the apex is directed away from the source of light the stem bends upwards: so that in both cases the superior surfaces of the leaves on the apical part of the stem are as usual exposed to, and the inferior surfaces turned away from, the incident light.

If the plants are kept in darkness for some time, almost the entire length of the stem becomes erect, and the leaves face more or less irregularly. If such plants are exposed to very weak illumination from above, the stems remain horizontal, and the leaves present their superior surfaces upwards. Hence the radial stem of *Pellionia* is diaheliotropic and also apogeotropic; but its diaheliotropic irritability is very much stronger than its apogeotropic irritability, for quite weak diffuse daylight is a stronger directive agency than gravity is. The plant thus affords a very good example of a radial stem possessing marked diaheliotropic irritability.

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On the Structure and Reproduction of Cystopus Candidus, Lév.

BY

HAROLD WAGER, F.L.S.,

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With Plates XV and XVI.

IN the year 1887, while working in Dr. Scott's laboratory at South Kensington, I undertook at his suggestion an investigation into the structure and mode of fertilization of *Cystopus candidus* (Lév.). A large number of observations and sketches were made, and a number of interesting facts determined, which tended to disprove the observations made by Fisch in 1885 as to the nature of the cytological structure and fertilization; but, owing to lack of material, I was at that time unable to complete the investigation. Since then, however, I have collected considerable quantities of fresh material in various parts of the country, and am now able to give a fuller account of the karyology and fertilization of this plant.

By the application of the new methods of study initiated by the introduction of the paraffin-method to botanical technique, by the improved methods of staining, and the use of the beautiful apochromatic lenses of Zeiss, a great

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impetus has been given to the study of the cytology and minute structure of the lower forms of plants, especially of the Fungi. Extremely interesting results have already been obtained, but the difficulty of prosecuting researches into the minute structure of parasitic Fungi is very great, mainly owing to the small size of these plants and their component parts. The work of staining sections is very difficult and demands much patience, owing to the great care which has to be taken in the application of the reagents and stains necessary to bring out the various details of structure, and still greater difficulties arise in the interpretation of the structures observed. To this must be due the conflicting statements made by various observers concerning the structure and development of the sexual reproductive organs in *Cystopus* and allied plants.

I am very pleased, therefore, to be able to record here that Mr. Trow has independently observed many of the facts mentioned in this paper, and in a letter to me states that 'the results so far obtained by me are, so far as I can judge from your descriptions, concordant with your own. In particular, I have no doubt as to the structure of the nucleus (excepting possibly the presence of a nucleolus which I have not observed), the number of nuclei in the oogonia, the number found by me averaged ninety-one, and the uninucleate character of the oosphere.'

Cystopus candidus is found in large quantities on various Cruciferous plants, especially on the Shepherd's Purse, *Capsella Bursa-pastoris*. It is difficult to find a patch of this common plant which does not show some of its members covered with the white spots and swellings so characteristic of the appearance of the Fungus, although it may easily be mistaken for *Peronospora parasitica*. It occurs on many other plants of the same order however, and Mr. Massee has been good enough to give me a list of the thirty-four species on which it has been found up to the present time.

It has a very wide geographical distribution, being found, as Mr. Massee informs me, in Europe, Asia, South Africa,

Ceylon, Victoria, New Zealand, Brazil, Cuba, the United States, Canada, the Falkland Islands, Surinam, and British Guiana.

The number of British species in addition to *C. candidus* is not numerous. In Massee's 'Phycomycetes and Ustilagineae' the following are given:—

C. Tragopogonis, Schroet.

C. Tragopogonis, var. spinulosus (C. spinulosus of De Bary).

C. Lepigoni, De Bary.

De Bary was the first to give us an account of the life-history of the plant, although, in the light of our recent knowledge, it is wanting in completeness. As his description is exceedingly clear and interesting however, I propose to give here a short account of the observations which he made in following out the life-history on fresh specimens.

The Fungus is commonly known as White Rust, and appears as spots or pustules on all parts except the roots and seeds. It attacks especially the stems and seed-capsules. It causes generally a large amount of hypertrophy of the organ attacked, which is sometimes visible even before the white patches are seen[1]. The filaments of the mycelium penetrate the tissues of the plant in the intercellular spaces. The walls are composed of cellulose, and are often thick and gelatinous. Small globular haustoria are produced frequently and penetrate the walls of the parenchymatous cells of the host, and come to lie just inside the cell-wall. They are spherical bodies about equal in diameter to the filaments of the mycelium or not so large. They are at first full of protoplasm, but later they contain only a watery fluid, and their membrane becomes thick and highly refractive, which makes them appear on a cursory examination like grains of starch. These organs are never wanting, and are so characteristic that one can often determine the presence of the mycelium by means of them.

The oogonia are formed by terminal or intercalary swellings

[1] I have often found such swellings to contain numerous sexual organs. H. W.

on the mycelium. Branches of the mycelium which do not carry oogonia apply their obtuse extremities against the oogonium, swell up and are cut off to form the antheridia. When the oosphere is formed, the antheridium puts out a narrow and erect tube from the middle of its face which is pressed against the oogonium, which perforates the wall of the oogonium, and traverses the peripheral protoplasm towards the oosphere. As soon as it touches the oosphere it ceases to elongate; the oosphere becomes surrounded by a cellulose membrane and takes a regular spheroidal form. The tube put out by the antheridium must be regarded as a fertilizing tube, but it is remarkable that it never opens, effecting fertilization by contact only; until the maturation of the oospore the antheridium retains the aspect which it presented at the moment of fertilization.

The membrane of the oospore becomes thickened: the epispore is derived from the peripheral protoplasm, which gradually disappears, and finally there remains only a quantity of granules suspended in a watery transparent liquid. At the period of maturity the epispore is a thickened membrane, very resistant, coloured yellowish brown and finely punctate. The surface of it is nearly always furnished with brownish warts, large and obtuse, sometimes isolated, sometimes confluent, forming irregular crests. The warts are composed of cellulose and are coloured deep blue by the known reagents, while the membrane which carries them retains its primitive colour. One of the warts larger than the others forms a sheath around the fertilizing tube. The endospore is a thick membrane, smooth and uncoloured, composed of cellulose; it encloses a layer of protoplasm finely granular, which surrounds a large central vacuole.

The conidial sporangia form zoospores. When sown in a drop of water so that they become soaked, they absorb water rapidly and swell; an obtuse papilla is developed on one side, and vacuoles are formed in the finely granular protoplasm, which disappear later: fine lines of demarcation at the same time divide all the protoplasm into five to eight polyhedral

portions, each of which shows in the centre a small feebly coloured vacuole.

Some minutes after the division, the papilla swells, opens, and the zoospores are pushed to the outside through the opening one by one, without giving the least sign of movement; they take a lenticular form, and group themselves before the opening of the sporange in a globular mass. They soon commence to move, vibratile cilia show themselves and the whole mass is set oscillating, the zoospores ultimately become free and move away through the liquid. The zoospores are of the form of a plano-convex or slightly concavo-convex lens. Under the plane or concave surface is a disc-like vacuole, and the cilia are attached on the edge of the vacuole, a short one in front, a long one behind, both on the same side. The generation of zoospores in the sporangia commences in 1½ to 3 hours after being placed in water. They appeared always in sporangia recently formed, or which had been kept a month and a half. But sporangia which were kept longer than that would not produce zoospores, and did not develop in any other way.

The oospores only change after a repose of several months. The oospore becomes a large sporange: placed in water the epispore bursts and the endospore protrudes. It contains a mass of protoplasm in which are to be seen several large vacuoles, which are constantly changing in form and volume. Soon this fluctuation is stopped, and in an instant the whole protoplasm is divided into polyhedral portions similar to the zoospores produced in the conidia. The endospore swells up to a much larger size and ultimately the zoospores are grouped in this vesicle in the form of a globular mass, which offers nearly the same phenomenon as in the case of the ordinary zoospores. Finally the spores separate from one another; for some minutes they swarm in the vesicle, then this bursts and disappears and the zoospores become free in the surrounding water.

The number of zoospores thus produced is very considerable; it is almost impossible to count them, but one may fairly

estimate them at about one hundred. They are similar to the ordinary zoospores. Their movement in the water goes on for two to three hours. Then they slow down, the cilia disappear, the spore becomes immovable, takes a globular form and is surrounded by a cellulose membrane. Then a thin tube is put out, curved or straight, which attains a length of two to six times the diameter of the spore on the object-glass. The apex of this tube is swollen, and all the protoplasm of the spore gradually passes into it. There is never any further development of the tube on the object-glass. The tubes never penetrate roots. They enter only through the stomata on leaf or stem. When a drop of water containing zoospores is placed on the surface of the leaf of a cruciferous plant, the spores will be found after the lapse of some hours fixed on the stomata, their movement having ceased. A germ-tube is produced on the side of the spore nearest the pore, which it enters immediately, and plunges the swollen extremity into the air-cavity of the stoma. The spore never passes through the stoma.

No further development of the tube takes place, however, and no mycelium is formed. It is only in the case of germ-tubes which enter the cotyledons that a mycelium is produced which spreads as the plant grows, and ultimately produces the characteristic white spots and pustules.

Literature.

The phenomenon of the formation of zoospores was described by Prévost as early as 1807. He describes them in a species found on the cabbage, which is evidently *C. candidus*. His description is given very clearly and with much detail, and there is no doubt that he had under observation the formation of zoospores in this plant.

L. R. and Ch. Tulasne in their memoir on the Ustilagineae and Uredineae ('47) refer to *C. candidus* (*Uredo candida*), and point out that the epidermis underneath which the

spores are immediately placed is often only slightly raised and irregularly torn by their development, and its projecting torn fringe surrounds the group of spores as a sort of indusium.

Léveillé ('47) founded the genus *Cystopus* with the following characters:—Receptacle composed of very small irregular cells, forming a sort of disc covered with cylindrical vesicles, terminated by several spores arranged in a chain. The spores are spherical or cubical. The following species were placed in the genus:—*Uredo candida* (Pers.), *U. cubica* (Mart.), *U. Portulacae* (D. C.), *U. Bliti* (Bivon.), *U. floriformis* (Mérat), &c.

Berkeley ('48) gave a short account of the structure of the mycelium and the sporangia of *Cystopus candidus*, illustrated by four figures, in which the moniliform character of the arrangement of the spores is described. This account was anticipated and confirmed by Léveillé.

L. R. Tulasne ('54) gave a description with figures of *C. Portulacae*, Lév. (*Uredo Portulacae*, D. C.). The mycelium is shortly described and its mode of ramification. The method of formation of the sporangia in *C. Portulacae* and *C. candidus* is described and good figures are given in Pl. VII, Figs. 1–5 of the appearance of the mycelium and spores of *C. Portulacae*. He states, however, that there are two kinds of spore developed in *C. Portulacae*—(1) shortly cylindrical spores, united into long chains, form the larger number; they have a smooth cell-wall and are sometimes nearly colourless, sometimes tinged with brown, and (2) spores with a spherical and trigonous form, larger, and tinged a yellowish brown which is always more or less deep. They are developed at the apex of the chain and are the first spores formed therefore. In *C. candidus* the spores are nearly always similar to one another, but sometimes one distinguishes larger ones with a manifestly three-sided form. The dimorphism which is very evident in *C. Portulacae*, is also present to a less degree in *C. candidus*. He was not able to confirm Prévost's observations as to the zoospore formation, but states that

germinate in six to ten hours, but he had never seen the other spores germinate, and once only had he observed a spore of *C. candidus* to germinate. In germination a filament of uniform diameter is developed.

Hoffman ('59) supported Tulasne's observations on the development of the sporangia, and stated that he believed he had seen the direct germination of spores of *Cystopus*, and was unable to accept the account given by Prévost.

De Bary ('60) confirmed Prévost's observations by observations on *C. candidus* and *C. cubicus*, and pointed out that the spores are really sporangia and produce in *C. candidus* from five to eight spores, and in *C. cubicus* from eight to twelve. He was not able to confirm Tulasne's observations as to the direct germination of the spores.

De Bary's larger memoir, which has already been quoted, appeared in 1863, in which the sexual organs were for the first time described.

Cornu ('72) in his Monograph on the Saprolegnieae referred to the growth of the cell-wall in *Cystopus*, and pointed out that at first the membrane of the oospore is thin and the contour perfectly regular. Later it is composed of a thin exospore and a thick endospore, and by examination of oospores of different ages it is seen that the external diameter increases in size while the internal one diminishes. There is, therefore, growth in two directions, centrifugal and centripetal. He objects to De Bary's observation that the epispore is formed from the periplasm, and points out that he has omitted to note the decrease in the internal diameter of the spore, which is even more easily seen than the increase in the external diameter. He regards it as more logical and more conformable with what is known as to the growth of cell-wall in other cases, to admit that the oospore formed at the expense of a part of the cellular protoplasm should be nourished afterwards by the remainder which it first of all absorbs. He very pertinently asks why, if the periplasm has the power of secreting a wall without being elaborated

previously, it does not deposit a layer also on the wall of the oogonium. If there is a necessity for elaboration, it would be more in accordance with what ordinarily occurs that it should be previously absorbed by the spore and afterwards be used up to form the external layers as well as the internal, and he therefore suggests as more probable that the epispore is formed by growth from the internal protoplasm of the spore.

In the oospores of *C. Bliti* the endospore is very thick, and is formed of concentric layers, which are coloured intensely by chlor-zinc iodide, while the intermediate spaces remain uncoloured. These layers are two or three in number. The upper one is moulded exactly in the crests of the epispore, and is coloured strongly blue-violet. The epispore is not coloured and is a brown encrusted layer.

In 1879 Schmitz gave an impetus to the study of the cytology of the Fungi by his memoir on the nuclei of the lower plants. Although he does not actually deal with *Cystopus*, he describes the presence of nuclei in *Peronospora calotheca*, and states that he has been able to observe them in many other members of the Peronosporeae.

Büsgen ('82) gave an interesting account of the formation of the zoospores in the conidia. He pointed out that the protoplasm of the conidia of *C. candidus* contains but few granules as compared with *Phytophthora*, in which the granules are more numerous. He evidently refers here to the nuclei which, as is now known, are much more numerous in the conidia of *Peronospora* than in *Cystopus*. His description of the formation of the zoospores is very carefully done, and I give here a short account of his observations. He points out that from one to one and a half hours after being placed in water, the sporangium becomes flask-shaped. Vacuoles of various sizes appear, and between them dark granules which become arranged in more or less regular lines. The protoplasm tends to contract away from the neck of the sporangium and from other parts of the wall, leaving clear spaces filled with a strongly refractive substance. The vacuoles unite together

and finally disappear. The protoplasm swells up again and becomes divided into spores by lines of granules which soon become converted into continuous cell-walls. Each of the spores thus produced contains a clear spot as already described by De Bary. The spores then escape from the sporangium, remain in connexion with one another for a short time, and then swim away.

Zalewski ('83) described the formation of the sporangia. The rounded summit of the basidium becomes superficially constricted and swells up until it attains the size of the basidium. At the level of the constriction a thin layer or border of cellulose appears, which grows gradually towards the centre and forms a transverse partition. When this division wall has attained its definite thickness, it divides into three layers, one of which belongs to the basidium, and another to the sporangium. These are separated by a third, which becomes gelatinous. This jelly-like membrane colours rose-yellow with iodine and sulphuric acid; the membranes of the sporangia and basidia are coloured blue. This jelly increases in volume and acquires its largest development when the sporangium is individualized. It becomes gradually resorbed as the sporangia develop, until only a very small portion is left between them in joining them together. On the addition of a small quantity of water the jelly is entirely dissolved and the sporangia become free. The jelly is also capable of absorbing moisture from the air, and is thus able to swell up and set the sporangia free. The chain of sporangia is now surrounded by a thin membrane which grows in and gradually separates them by forming the gelatinous substance which connects them together as De Bary at first thought.

In the same year ('83) Zalewski, in another memoir, gives an account of his observations on the structure and life history of *Cystopus*, in which he describes the formation of the conidia (sporangia) again, and the structure and development of the oospore as observed on living specimens. In connexion with the formation of zoospores he makes the interesting

observation that they appear in summer in from two to three hours, but in autumn not until one to two or three days after the sporangia have been placed in water.

His observations on the oospores were made on a *Cystopus* found on the undersides of the leaves of *Cirsium arvense*. He kept sections of the leaf with oospores in a damp chamber, but found that the culture could not go on longer than three to four days, as after a longer time the oogonia became degenerated. In addition therefore to observations made in this way, living oogonia in various stages of development were observed and drawn at the same time, and the author concludes that the exospore is undoubtedly formed from the periplasm. As he could only keep them three days, he was obliged to begin each third day with different stages, so that, as he says, his observations are probably only approximately correct. Once only was he able to observe a spore for five days.

In the oogonium, soon after it has become filled with protoplasm and cut off from the mycelium, the oospore is differentiated and is surrounded by a thin cellulose membrane. In three days more the wall has trebled or quadrupled itself in thickness by a deposition of the periplasm. The periplasm, shortly after the formation of the membrane around the oospore, has a dense, frothy, reticulate appearance. The vacuoles surrounded by this reticulate protoplasm are somewhat angular. They grow considerably while the periplasm slowly streams into the meshes towards the wall of the oospore in order to form the exospore. The meshes break down here and there; the vacuoles unite with one another, and at last the oospore remains suspended to the wall of the oogonium by means of a few protoplasmic strands. These break through also, and the whole periplasm, or a great part of it, falls down upon the wall of the oospore, whilst the remainder clings to the wall of the oogonium or floats about in the sap and slowly becomes brown and dies. At first the wall of the oospore is formed uniformly throughout, but when the streaming of the protoplasm becomes more rapid so

that it spreads unequally, then the wall of the oospore becomes unequally thickened, and the ridges may become so pronounced as to look like warts. The fertilizing tube of the antheridium also becomes surrounded by a thick exospore.

The exospore begins to take a brown colouration when the projections are yet small; it is already quite dark brown when these are uncoloured, the latter requiring a longer time to attain to the brown colouration.

So far as the author's observations go it takes probably from eight to ten days from fertilization to the ripening of the oospore.

He does not accept Cornu's observations as to the structure of the wall of the oospore. The endospore consists of pure cellulose. The exospore is cuticularized. In the majority of cases, as in *C. candidus*, the exospore consists of four layers.

The thickness of the separate layers differs in the different species. The inner layer is thin homogeneous and cuticularized—yellow or brown with iodine and sulphuric acid. The next layer consists of cork, but is seldom homogeneous (as in *C. Convolvulacearum*) and usually finely granular; it is formed of thin, closely packed, round or angular rods, and in *C. candidus* is the thickest layer. In all other species except *C. sibiricus* it is very thin, and is completely wanting in *C. Portulacae*, *C. Amarantacearum* and *C. Bliti*. The third layer is the cellulose-like layer. It is most strongly developed in *C. sibiricus* and *C. Convolvulacearum*. In *C. candidus* it forms its thick upper ridges without spreading out into an unbroken layer, and in *C. cubicus*, *C. Lepigoni* and *C. Bliti* it only forms the inner part of the projecting ridges and warts.

Outside this cellulose layer is a fourth (or third) thin, commonly dark brown cuticularized layer. There is no distinct line of demarcation between this and the other cellulose layer however, and it appears to be merely the cuticularized outer portion of the latter.

The cellulose layer is dissolved by long treatment with sulphuric acid. This is best seen in *C. candidus*, where the projections of the surface then appear quite hollow.

The appearance of the contents of a ripe oospore of *C. candidus* is as follows:—The large central space contains a globule of oily matter about half to two-thirds the diameter of the lumen. It is surrounded by a coarsely granular protoplasm, the outer layer of which contains clear, round spots in variable number, three to four or more.

In 1885 Fisch gave an account of the structure and fertilization in species of *Pythium*, and stated that, according to his incomplete observations, *Cystopus* appears to behave in the same manner. In the young oogonia of *Pythium* there are usually from ten to twenty nuclei. In the formation of the oosphere these fuse together to form a single large nucleus. In the antheridium he always found a single nucleus, but thinks that this may be due to the fusion of several. The nucleus of the antheridium passes into the oosphere with the gonoplasm, and disappears there with the egg-nucleus.

In 1888 Chmielewsky gave a description of the process of fertilization in *C. candidus* which is very different from that given by Fisch. The protoplasm of the young oogonium is net-like, and the knots of the net, which consist of granular collections of plasma, stain strongly. Fisch must (says the author) have mistaken these knots for nuclei. In reality the young oogonium contains only one nucleus, and that is parietal. It is usually large, and has the form of an ellipse, in one focus of which the very small nucleolus is generally placed. The nucleus is very poor in chromatin, and stains feebly. The nucleus passes later into the centre of the oogonium, and the distinction between epiplasm and gonoplasm becomes visible. Shortly before fertilization the nucleus contracts a little and becomes strongly stainable (but even now is larger than the nuclei of the vegetative hyphae). The antheridium contains one nucleus only, which is similar in size and staining properties to the nucleus of the oosphere, a peculiarity which is to be noted. The passage of the male

nucleus and gonioplasm takes place through a fertilizing tube, which often penetrates deeply into the oosphere. The author observed the two nuclei in the oosphere, and found that they finally become fused. The ripe oospore contains only one nucleus.

Dangeard ('90) gives an account of the histology of *C. candidus* Pers., and *C. cubicus* Strauss.

In *C. candidus* the haustoria are very small, and contain only one nucleus or none at all. The nuclei in the mycelium are fairly well spaced. The conidia (sporangia) contain a hyaline protoplasm, and a number of nuclei, five to seven, as many as the number of zoospores which the conidia form. It appears that the nuclei all come from the mycelium and there is no division in the basidium. After the formation of a number of conidia, the membrane of the basidium becomes thick, and only two to four nuclei are left in it.

The oogonia are generally produced at the ends of the branches, but may also be intercalary. They contain numerous nuclei which appear to possess nucleoli. The structure described by Chmielewsky as a single large nucleus is considered by Dangeard to be an oil-globule. After the differentiation of the protoplasm of the oogonium into oosphere and periplasm, a corpuscle is found in the centre of the oosphere of a variable form and more or less stained. In the periplasm several small nuclei are to be observed. Later the ripe oospore contains a large corpuscle in the centre, which stains strongly. It is nothing else than an oil-globule which is found also in other oospores, particularly in the Saprolegnieae and Peronosporeae. Sometimes it is a sphere, sometimes a ring, sometimes an arc of a circle. If chloroform is allowed to act long enough it disappears, and there is no longer any colouration. Sometimes when the chloroform has not acted for a sufficiently long time it takes the form of a sponge.

The nuclei according to Dangeard are masked during the fertilization, and reappear again distinctly in the old oospore, in which from eight to ten nuclei can be observed placed in the layer of protoplasm between the globule of oil and the

endospore ; they resemble by their size and other properties those which have been seen in the vegetative organs.

He arrives at the conclusion that of the nuclei of the oogonium some remain in the oosphere, some in the periplasm ; to the nucleus in the oosphere are probably added those of the antheridium, although he found it impossible to observe their passage. In any case the ripe oospore contains about twenty nuclei. The oil globule grows little by little until it forms the large central globule of the ripe oospore.

He was not able to observe any fertilization, and did not observe the division of the nucleus in the oogonium nor in the oospore. He accepts Zalewski's observations on the structure of the wall of the ripe oospore.

Mangin ('91) describes the disarticulation of the sporangia in *C. candidus*. When a sporangium is about to form, a thin ring of callus appears near the apex of the basidium. This gradually grows inwards until a transverse partition has been completely formed, of a convex or conical shape, the concavity being towards the sporangium. The cellulose wall in contact with this transverse partition at the edge becomes resorbed, and a constriction is produced by an apparent contraction and thickening of the callus partition. This proceeds rapidly, and ultimately the callus forms a little cup-like formation at the base of the sporangium, by which the latter is attached to the basidium. The author was not able to observe the division of this layer into three as observed by De Bary and Zalewski. Ultimately the callus-like substance becomes reduced to a short cylindrical mass connecting the sporangium to the basidium, and the cellulose wall both of the basidium and the sporangium ultimately extends all round on both sides of the callus. The portion of callus left between them slowly acquires the property of dissolving in water by which the sporangia are set free.

When the basidia have formed a number of sporangia their activity ceases, and as the hyphae are interrupted here and there by masses of callus which stop communication with the

other parts of the mycelium the formation of sporangia ceases.

In 1892 I gave a description of the structure of the nuclei of *C. candidus*, and pointed out the multinucleate nature of the oogonium and antheridium. Like Dangeard I was, however, unable to observe fertilization or the fate of the nuclei in the oosphere, and agreed with him that Fisch had mistaken the central oil-globule for a nucleus. Since then I have been able to disprove this, as will be seen later.

Peglion ('93) in a paper on the hypertrophy of the tissues produced by *C. candidus* on *Raphanus Raphanistrum*, points out that large accumulations of starch are produced where the conidia pustules are formed. The epidermal cells and the cortical parenchyma are increased in size, the former especially tangentially, and the vascular bundles are modified.

Macallum ('95), in his paper on the distribution of assimilated iron in animal and vegetable cells, shows that a substance in which iron is firmly held appears to be a constant constituent of the nucleus and of the cytoplasm of non-nucleated cells and those possessed of apparently rudimentary nuclei. He says that in *Cystopus candidus* the whole of the protoplasm in the mycelium and gonidia, except the mature gonidia, is chromophilous, that is, contains chromatin. The nuclei in the mature gonidia are of the more regular form, but in the mycelium are chiefly, if not wholly, small masses of chromatin substance, like those forming the 'nucleoli' in the abjointing gonidia. Mitotic phases were not seen.

The disposition of the assimilated iron corresponds closely with the distribution of the chromophilous substance. The small masses of chromatin and the nucleoli gave abundant evidence of its presence, the remaining portion of the nucleus in the case of the latter containing very little, relatively less than the cytoplasm.

In the subsequent development of the abjointed gonidia, the nuclei appear to take up from the protoplasm all, or nearly all, the substance containing iron, and with this the character of the nuclei seems to change. The nucleoli first of all are

converted into fine granules distributed through the nuclear cavity, and finally in the mature gonidia the nuclei appear to be simply more or less homogeneous masses of iron-holding substance, while the cytoplasm does not contain a trace of the metal.

In 1895 Istvánffi observed the multinucleate character of the vegetative hyphae in *Cystopus Portulacae*, *Peronospora Ficariae*, *P. Chlorae*, *Phytophthora infestans*, &c., and also pointed out that the conidia contain several nuclei.

He investigated also the nuclei of the sexual organs in *Cystopus Portulacae*. The material was hardened in alcohol, by which the fatty matters were dissolved out.

The protoplasm, as it passes into the swollen end of a thread to form the oogonium, possesses a peculiar structure. It streams into the oogonium from the hyphae, and exhibits at the base of the oogonium a radiating structure which gradually passes over into a network. In the meshes of the network the nuclei are found; only occasionally are nuclei to be found in the streaming portion. The oospore is formed out of the network of protoplasm, and appears as a large spherical body inside the oogonium; its contents are formed of a quite dense protoplasm, and the network structure is no longer visible. The space between the oosphere and the wall of the oogonium contains epiplasm, which forms the brown, net-like thickening of the oosphere. The formation of the oosphere seems to take place only after fertilization, for the antheridium is already attached to the oogonium in the early stage, when its protoplasm still shows the reticulate structure. It contains many nuclei, and empties itself before the oosphere is rendered visible. It is probable that fertilization takes place by the fusion of the male and female nuclei.

No reference is made to the separation of the nuclei in the formation of the oosphere, as to whether one or several nuclei remain in the oosphere, but in his explanation of Fig. 25--an oogonium with oosphere in a young stage—he states that 'a part of the nuclei remain in the epiplasm,' and figures also a large number of nuclei in the oosphere.

METHODS.

I have always found it necessary to make a preliminary investigation of the piece of stem infested by the Fungus in order to determine the presence of the sexual organs. Sections were taken here and there and examined, and, when a suitable region of the stem had been discovered, it was cut up into quite small pieces not more than about $\frac{1}{16}$ to $\frac{1}{8}$ inch long. Each piece was halved longitudinally, which allows the satisfactory penetration of the re-agent, and was then placed in the fixing fluid. As a fixing and hardening re-agent, I have found corrosive sublimate ($HgCl_2$) the most useful and successful. This is used in saturated solution, and the pieces of stem are placed in it for from one or two hours to a whole day. I have also used absolute alcohol and chrom.-osmium acetic acid, but corrosive sublimate is the most satisfactory in every way.

The pieces of tissue are then well washed in water, transferred to 30 % spirit, 50 % spirit, and 70 % spirit, about two or three hours in each, and finally transferred to 90 % spirit.

The tissues may now be stained *en bloc* and embedded in paraffin; or they may be first embedded in paraffin and the sections stained on the slide. I have tried both methods, and found the latter the more successful, as it allows the greatest amount of latitude in determining the extent to which it is advisable to allow the stain to act, and the whole operation of staining and clearing can be controlled under the microscope.

For embedding previously to staining, the tissues are transferred to absolute alcohol for half an hour or so, then to a mixture of alcohol and xylol, and are finally placed in melted paraffin, where they remain for an hour or so.

The sections were then cut in the ordinary way by a Cambridge rocking microtome. The ribbon was cut up into suitable lengths, which were mounted on the slide in the following way. The pieces of ribbon were first floated on the surface of warm water, which causes them to spread out perfectly flat. A piece was then floated on to a slide, the

superfluous moisture sucked up with blotting paper and the slide allowed to dry at the ordinary temperature of the room or in a gentle heat. There is no need to use any kind of cement if one is very careful to allow the drying to be complete. The paraffin is then melted and dissolved in xylol. From xylol the slide is transferred to absolute alcohol and then to 90 % spirit, where it may remain until a convenient time for staining.

The methods of staining adopted have been various, but the one which gives most beautiful and satisfactory results is a slight modification of one kindly communicated to me by Prof. Hartog and devised by him. The solutions used are those recommended by Hartog, somewhat modified for my purpose, viz.

1. 50 % spirit, 4 vols.; glacial ac. ac. 1 vol.

2. To solution 1 enough nigrosin is added to make it opaque in the bottle and transparent in a half-inch layer.

3. To solution 1 enough nigrosin is added to make it blue, but transparent in a layer two inches thick.

4. 50 % spirit, to which a small quantity of 2 is added to make it quite light blue in colour.

5. Mayer's alcoholic solution of carmine.

The sections are placed for about five to ten minutes in the mordanting solution 4.

They are then placed in Mayer's carmine for a few minutes until they become stained distinctly red, then washed in 30 % spirit and placed in solution 3. If a deep stain is required solution 2 must be used.

The sections remain in solution 3, called by Prof. Hartog a toning solution, until the required effect has been produced. The exact stage at which the operation should be stopped can be easily determined by observing the sections under the microscope. In order to produce a very clear and well-differentiated stain, I next transfer the sections to solution 1, in which a good part of the stain becomes washed out, leaving the nuclei beautifully differentiated and every part clearly shown up. It requires considerable practice to enable one

to judge of the length of time which these stains should be allowed to act, but I have found that the best results are obtained by a very short stay in carmine. I have occasionally found it useful to differentiate in Hydrochloric acid alcohol.

The sections are then transferred to 70 % spirit, methylated spirit and absolute alcohol. They are next transferred to xylol or carbolized xylol and mounted in xylol balsam.

I have used in the investigations the apochromatic lenses of Zeiss; 4·0 mm., apert. 0·95; 2·0 mm., apert. 1·40, and oculars 8, 12, and 18. In order to obtain the best illumination possible, I used a high-angled oil-immersion condenser, and recently the Welsbach Incandescent light, which surpasses anything I have previously worked with.

Structure of Mycelium and Haustoria.

The mycelium is not so easily seen, and its structure is more difficult to observe than is the case in *Peronospora parasitica*, which commonly occurs with *Cystopus* on the same host-plant, and it may be easily overlooked unless it is present in abundance. The hyphae are irregular in size and outline, according to the shape of the intercellular spaces through which they pass, and branch frequently. The branching is very irregular, depending upon the presence of the intercellular spaces, and it occasionally happens that when a branch reaches a larger intercellular space than usual it expands considerably and produces branches radiating from it in all directions. The protoplasm contained in the hyphae is granular, and loosely arranged in a network with numerous meshes. It is sometimes very scanty in amount, and forms just a thin layer on the wall of the hypha with a few strands stretching across here and there. It is on the other hand occasionally very dense indeed, completely filling the lumen of the hypha. This occurs where probably active growth is taking place, and is often found in those portions of the mycelium on which the basidia are borne. In the normal condition the protoplasm stains reddish blue in the carmine-

nigrosin stain; where it is very dense, however, the red stain predominates. The haustoria are small, spherical projections produced here and there on the hyphae, occasionally in considerable numbers. They often indicate the presence of the mycelium before this has been observed, and it is often very difficult to see their connexion with the hyphae upon which they are borne owing to the small size of the connecting tube. The protoplasm in the haustoria takes always a distinctly blue colouration in the carmine-nigrosin stain, with no tinge of red. In some cases it is very dense, stains deeply, and nearly completely fills the cavity; in others it forms a peripheral layer of deeply stained granules around a central vacuole. I have never been able to observe nuclei in the haustoria, although Dangeard ('90) states that they sometimes contain one. This is perhaps interesting from the point of view of the function of the nucleus, as showing that the presence of a nucleus in the haustoria is not absolutely necessary in order to enable them to properly perform their function of absorption of food-material. It must be pointed out, however, that as these haustoria always remain small, and that the whole of their protoplasm therefore is not far removed from the influence of the nuclei of the hyphae upon which they are formed, there may be no necessity for the actual presence of a nucleus in them. In *P. parasitica*, where the haustoria are very large and sometimes nearly completely fill the cells of the host-plant, they always contain numerous nuclei. The protoplasm of the haustoria in these stained sections is generally shown slightly contracted away from the wall equally all round, which gives them a very characteristic appearance, as if they possessed a very thick cell-wall.

The nuclei of the mycelium are generally very loosely arranged in the protoplasm, but where the protoplasm is abundant and where probably active growth is taking place, the nuclei are more numerous. In the former case the structure of the nuclei can be easily observed, but not in the latter. In the resting condition each nucleus consists of a nuclear membrane, network, and nucleolus. The network

stains light blue, the nucleolus reddish blue. The network of the nucleus appears to be distinctly granular.

I have never been able to clearly observe nuclear division in the mycelium, although I have constantly seen appearances which gave me the impression that they were due to nuclei in a state of division. But the nuclei are so small and the amount of chromatin so insignificant that, judging by the difficulty I experienced in making out nuclear division where the nuclei are much larger, as in the oogonia, it will, I think, be extremely difficult to satisfactorily observe it here.

Formation of Gonidangia.

The gonidangia are produced on basidia, which are formed in large numbers just underneath the epidermis of the stem, leaves, and fruits. Protoplasm and nuclei pass into the basidia from the mycelium. The mycelium is very much branched and abundant in the region where the basidia are given off, and the cell-walls, especially in the later stages, are very much thickened and stain easily in haematoxylin. No fusion of nuclei has been observed in the basidia, such as has been described in the Basidiomycetes by Rosen, Dangeard and myself, and in the Asci of Ascomycetes by Dangeard and Harper. This is interesting, and tends to emphasize the different nature of the basidia in *Cystopus* as compared with those in the Basidiomycetes and Ascomycetes, which are to be regarded as exhibiting a kind of sexual fusion of nuclei (as has been shown by Dangeard, '95 ; Harper, '95 ; and myself, '93). In *Cystopus*, the formation of the conidia is preceded neither by fusion nor by division. Some five to eight nuclei, together with a quantity of protoplasm, accumulate at the apex of the basidium, which is then cut off by a transverse wall to form the gonidangium, as shown by Zalewski ('83) and Mangin ('91). The four to eight nuclei remain undivided, and each one becomes the nucleus of a zoospore. Each nucleus is surrounded from the beginning by a quantity of dense granular protoplasm, which tends to render it invisible,

as the protoplasmic granules stain deeply. The nuclei possess a nuclear membrane, nucleolus and nuclear network, and are perhaps a trifle larger and more distinct than those in the mycelium. The protoplasm, both in the basidia and in the sporangia, stains deeply with all the ordinary nuclear stains. The formation of the zoogonidia has been described by Prévost ('07), De Bary ('63), and Büsgen ('82).

Macallum's observations ('95), already quoted, appear to accord very well with my own as to the distribution of stainable (iron-containing) substance. But I have been able to observe that the small chromatin-masses in the hyphae are the nucleoli of the nuclei. No figures are given by Macallum of the mycelia and haustoria, and it would be interesting to know whether the author had been observing the mycelia of *P. parasitica*, in which the nuclei are very rich in chromatin-substance and the haustoria very large, or the mycelia of *C. candidus*, in which the nuclei do not contain so much chromatin and in which the haustoria are very small, as both fungi commonly occur on the same host-plant and the mycelium of one might be mistaken for that of the other.

Formation of the Sexual Organs.

The sexual organs, described by De Bary in 1863, are found in considerable abundance in favourable specimens. It is impossible to say what induces their formation, or at what stage in the life-history of the fungus they appear, or under exactly what conditions. So far as I have been able to observe, a large supply of nutrient matter, such as occurs in the more succulent parts like the stem, is a general concomitant of their appearance. It is not safe to trust too much to the abundance of the conidia on the surface of the stem, or to the decay of the stem, leaves, or fruits, as an indication of their occurrence. I have often found stems in an advanced state of decay, completely covered with the old basidia, to contain no sexual organs. On the other hand,

I have often found portions of the stem, very much hypertrophied, but with few or no basidia and gonidangia, to contain them in abundance. One may look for sexual organs in vain in some localities and find them plentifully in others, and one may find them in stems the surface of which is almost completely covered with the asexual reproductive organs of *Peronospora parasitica*. They may be accompanied by much hypertrophy or by very little, with many asexual organs or with very few. I have generally found it a pretty certain indication of the presence of sexual organs if the portion of the stem on which they are suspected to occur is brittle, and easily broken when slightly bent. Where they do not occur the stem may be bent double before it breaks. In cutting sections by hand, one is also able to judge fairly easily whether sexual organs are present or not. When present, the sections come off beautifully smoothly, and without the least suspicion of tearing or toughness ; but if not present, there is a larger resistance to be overcome, and unless the razor be in good condition a certain amount of tearing is the result. Generally speaking the sexual organs are to be found more easily towards the end of the season than at the beginning.

In many cases, and perhaps in the most favourable condition for observation as a rule, that part of the stem in which they are to be found in the early stages of their development is coloured a dark reddish purple and is somewhat swollen. When the oospores are mature, the stem becomes brown and rotten, and the tissues almost completely broken up. In the earlier stages of their formation, although they may be found in great abundance in some parts of the stem, there is no apparent degeneration visible in the cells of the host-plant such as appears later. The cells contain protoplasm, nuclei, and chlorophyll grains, and present a normal appearance, except for the presence of haustoria which are to be found lining the cell-wall in some cells in considerable numbers. Peglion ('93) has given a very careful account of the action of the fungus upon the tissues of the host-plant.

In the formation of oogonia, protoplasm and nuclei pass from the mycelium into an expansion, generally produced at the end of a filament, in large quantities. The protoplasm appears to pass in very rapidly, and there is always a dense mass of it at the opening leading into the young oogonium, and radiating from this region into the oogonium are striae indicating the flow or streaming of the protoplasm (Fig. 4), a phenomenon which Istvánffi ('95) also describes and figures. The oogonium at this stage often presents a contracted or somewhat crumpled appearance, and the protoplasm is arranged in an irregular network in which the foam-structure visible at a later stage cannot be seen. As the nuclei pass into the oogonium they also present a crumpled, irregular appearance, looking very much like knots in the protoplasm (Fig. 4), and it would be very easy to mistake them for such, if it were not that in very carefully prepared sections the nuclear structure can in some cases be seen. When a sufficient quantity of protoplasm and nuclei have passed into the oogonium, a transverse wall is formed separating it from the hypha upon which it is formed: the oogonium immediately expands, as if it suddenly became turgid, loses its irregular crumpled appearance and becomes smooth, and, so far as the surrounding tissues will allow, spherical in outline (Fig. 5). The nuclei also regain their shape and are then seen to be spherical in outline and similar to those in the mycelium, or perhaps a little larger. Even now, however, in badly prepared sections, it would be perfectly easy to mistake them for knots in the protoplasm as Chmielewsky ('88) did. The protoplasm at this stage has a distinct foam-structure which is very clearly shown in well-stained preparations, and the nuclei occur more or less regularly spaced in the protoplasmic reticulum (Fig. 6). The nuclei possess a nuclear membrane, a small nucleolus, and a network which is, if anything, slightly more distinct than in the nuclei of the mycelium. This is probably due to changes which appear to be set up as regards the supply of nutriment as soon as the oogonium becomes delimited from the mycelium. The number of nuclei present

at this stage varies according to the size of the oogonium. I have counted, in different oogonia by an examination of serial sections, and by making allowances for nuclei cut in two, and so observed twice,—though the section is rarely of such equality as to deceive one in this way,—64, 75, 88, 97, and 115 nuclei, and numbers varying from 70 to 110 are common in all stages up to division[1]. The protoplasm and nuclei at this stage stain with a decidedly reddish tinge, similar, though not quite so red perhaps, to what is observed as they pass into the oogonium. Before very long, however, owing probably to changes in the supply of nutriment, both nuclei and protoplasm become more blue, the nucleolus only retaining its decidedly reddish tinge.

At about this time the antheridia attach themselves closely to the oogonia. They possess a number of nuclei varying from six to twelve or perhaps more. Both nuclei and protoplasm have the same structure and appearance as in the oogonia, and the changes in the appearance of the nuclei in an oogonium are accompanied by similar changes in the antheridium. The protoplasm is, however, denser than in the oogonium and renders observation of the nuclei correspondingly more difficult. Soon after the antheridium comes into contact with the oogonium a change takes place leading to the appearance of a granular, more or less homogeneous, mass of protoplasm just beneath the wall of the oogonium on the side nearest the antheridium (Fig. 5). This, as will be shown later, is the place where the receptive spot appears and is the first indication of it.

From this stage onwards considerable changes take place in the oogonium. The nuclei increase in size, the network becomes more distinct, and the protoplasm becomes more vacuolate and exhibits more clearly the foam-structure already mentioned (Fig. 6). These changes lead up to the division of the nucleus and the formation of the oosphere.

[1] Mr. Trow informs me in a letter that the average number found by him was ninety-one.

FORMATION OF THE OOSPHERE AND FERTILIZING TUBE.

In the denser and more granular protoplasm on the side of the oogonium nearest to the antheridium, already noticed, a hyaline space appears, from which a papilla with a deeply stained apical spot projects towards the antheridium, tending to bore its way through the wall of the oogonium and causing the wall to become somewhat thinner at this place. The protoplasmic contents of the antheridium appear to be contracted at first in front of this projection and a slight depression is produced (Fig. 6); but soon a dense granular mass appears in the antheridium, on the side nearest this projection, and a fertilizing tube is put out which penetrates the oogonium at this spot (Fig 10). This however does not take place until the formation of the oosphere has commenced. The receptive papilla, as we may call it, is very clearly located in the oogonium by the contraction of the protoplasm from all parts of the wall except at this point, where it appears to be securely attached, and remains so during the whole of the time required for the development of the fertilizing tube. While these changes are taking place the nuclei, both of the oogonium and antheridium, increase considerably in size. The network becomes more distinct and stains more deeply, so that its granular nature can be easily made out.

The differentiation of the oosphere now begins. The protoplasm contracts towards the centre into a roundish mass, connected to the wall of the oogonium by thick protoplasmic strands (Fig. 8). This central mass contains all the nuclei; none are to be found in the thick peripheral strands of protoplasm. It gradually becomes further differentiated into a central vacuolate and reticulate mass which stains with a reddish tinge, and an exterior peripheral layer of very dense non-vacuolate protoplasm, the periplasm, which stains with a bluish-red tinge. The whole of the protoplasmic contents of the oogonium outside the oosphere become finally condensed into this periplasm, with the exception of a few

strands which still connect it to the wall of the oogonium (Fig. 9). The nuclei at the same time undergo changes leading up to division. The nucleoli disappear and the network appears to divide up into chromosomes and contracts towards the middle of the nucleus in a very characteristic manner (Figs. 8 and 9). The network of chromosomes takes on a reddish tinge instead of a blue one and the nuclear membrane is very distinct. There can be no doubt, as will be seen later, that this contraction of the nuclear network, which gives the nuclei a very characteristic appearance at this stage, is preliminary to the division of the nuclei which takes place shortly afterwards. The majority of the nuclei are now to be found in the periplasm, and they gradually become more and more restricted to this layer. Meanwhile, however, nuclear division actually begins. Each nucleus divides into two, karyokinetically, and the appearance presented by an oogonium at this stage is shown in Fig. 9, in which a number of nuclei are to be observed in the periplasm which have undergone division, as well as nuclei in the centre still undergoing division. The various stages of division, so far as I have been able to observe them, are given in Fig. 28 (1 to 5), and are typically karyokinetic.

While the division of the nuclei is taking place, a dense, very deeply stained blue, mass of protoplasm appears in the centre of the not yet completely differentiated oosphere. This appears at first sight to be completely homogeneous, but on closer examination is found to be made of a dense mass of granules closely packed together and more or less sharply marked off from the rest of the protoplasm. This is probably what was described by Dangeard ('90) as an oil-globule, and was mistaken by Chmielewsky ('89) for a nucleus. Dangeard's observation that this substance disappears on soaking the tissue for a long time in chloroform, was probably due to the fact that it is not so apparent in some oogonia as in others, although it is generally present, even when casual observation of a thick section might lead one to the conclusion that it was absent. That it is not oil is proved by its structure and by the fact that oil is not present except

in minute drops at this stage, and that it will not dissolve even after a long soaking in alcohol, ether, and chloroform. It is of the same nature as the dense protoplasmic mass which appears in the fertilizing tube at the moment when it begins to grow, and is produced probably by an accumulation of stainable granules from the protoplasm. This dense mass of protoplasm can be observed in oogonia of all stages, such as are figured in Figs. 8 to 22. Shortly after its appearance, one of the nuclei produced by the division in the oogonium comes into close contact with it and gradually becomes more or less completely embedded in it. All the other nuclei pass to the periplasm, leaving this single nucleus in the centre as the nucleus of the ovum (Figs. 13 and 14). We may consider that at this stage the oosphere has become differentiated, although its cell-wall has not yet appeared. It is interesting to note that the wall of the oosphere does not form until just at the time when fertilization takes place, or shortly afterwards.

The Division of the Nucleus.

As already pointed out, the division of the nucleus in the oogonium is karyokinetic and is very similar to the normal process as described in the higher plants. The more the structure of the nucleus is investigated in the lower plants the more clearly does the fact become apparent that, instead of a much simpler and more rudimentary type of nucleus than that so well known in the higher plants, it appears to be almost exactly similar, the former differing from the latter in no essential respect, except perhaps as regards the presence of centrospheres. Indeed it appears to me that in some cases, certain details of structure come out more clearly in these lower plants than in the higher, provided a sufficiently good magnifying power be used. In any case I think we may fairly arrive at the generalization that simplicity of vegetative structure does not necessarily mean simplicity of cytological structure, and that it is probable that further

investigations will tend to bring the structure of the nuclei of the lower plants still more into accordance with those in the higher plants and animals. All the evidence we have of the details of nuclear division in the fungi strongly support this conclusion, and it may be useful here to give some short account of the observations which have so far been made bearing directly on this question.

Sadebeck ('83) describes nuclear division in the Asci of *Exoascus* as distinctly karyokinetic, and gives figures of nuclei in a late stage of division in which the spindle-formation is seen.

Strasburger ('84), in an account of the formation of the sporangia in *Trichia fallax*, describes a nuclear division which follows the ordinary karyokinetic method, and in a more recent paper ('94) he states that these nuclei each contain twelve chromosomes.

Fisch ('85), in his paper on the development of *Ascomyces*, gives a description of nuclear division in which he points out that at the commencement of the division granules appear in the nucleus, that this is immediately followed by the spindle-stage, and that in many respects the division resembles that which takes place in the higher plants.

Eidam ('87) describes the division of the nucleus in *Basidiobolus Ranarum* as indirect, and states that the details of the division can be brought into accord with those known in the higher plants.

Hartog ('89 and '95) regards the division of the nucleus in *Saprolegnia* as essentially a special case of karyokinesis, intermediate between direct division and the commoner process. According to him the nuclein-mass of the nucleus separates into four rods which form the nuclear plate. They undergo longitudinal division, so that the plate consists of eight rods, and finally these separate into two crescentic groups which become the daughter-nuclei.

In 1889 I was able to show that the division of the nucleus in the oogonium of *Peronospora* showed some details of karyokinesis, including the probable appearance of a spindle.

Rosen ('92) describes the process of nuclear division in the Fungi as not altogether indirect, and simpler than in the higher plants. He was not able to observe any of the details of spindle-formation, &c., and states that the smaller the nuclei the simpler their division. I have already pointed out ('93) that his observations on nuclear division in the Basidiomycetes are incomplete.

Gjurasin ('93) shows that, in the division of the nucleus in the Asci of *Peziza vesiculosa*, a spindle-figure appears with protoplasmic radiations at the poles, that the granules in the nuclear network pass to the equator of the nucleus and divide into two halves, which move very rapidly to the poles of the spindle to form the two daughter-nuclei.

Lister ('93), in his paper on the division of nuclei in the Mycetozoa, describes the division in the formation of the swarm-cells, and gives beautiful figures illustrating the various stages, which conform in general to what is known in the higher plants.

In 1893–4 I showed that in the Hymenomycetes we have a very clear case of karyokinesis in the basidia; spindle-figure, equatorial plate, protoplasmic radiations at the poles of the spindle, and probably centrosomes, being produced.

Harper ('95) describes and figures beautifully the nuclear division in *Peziza* and *Ascobolus*, which is seen to follow almost exactly the normal course.

Trow's description of nuclear division in *Saprolegnia* ('95) is obviously incomplete. He states that the nuclei divide directly in the germinating zoospores, but that in the oogonia the division is indirect. The nucleus contains one chromosome of irregular shape, which divides into two, producing two 'half-chromosomes.' He regards the division therefore as a reducing one. In a recent letter to me, however, he states that 'a long study of the work which has been done on the behaviour of the nucleus in gameto-genesis has led me to the conclusion that the network I have figured is a linin-network impregnated with chromatin, and the division figured

simply a very special case of ordinary karyokinetic division brought about possibly by the small size of the nucleus.'

Finally, Isatschenko ('95) describes a process of indirect nuclear division in the cells of the pileus and stipe of *Pholiota aurea*, Fr., in which the chromatin substance collects at the equator of the nucleus and divides into two portions which gradually approach the poles of the nucleus, where small round bodies (centrosomes ?) can be seen. The nucleus becomes constricted in the middle and two daughter-nuclei are formed.

In the process of nuclear division, as shown in the oogonium of *Cystopus* (Fig. 28, 1 to 5), the nucleus first of all loses its nucleolus (1) and the network becomes at the same time clearly visible and is seen to be granular. Its network then appears to be transformed into a number of granular chromosomes (2), which stain reddish, and among which a faintly stained blue network is to be seen. The appearance of the nucleus at this stage gives one the impression that the chromosomes are very numerous, but on counting them, as well as one is able, there appears to be about 12 or 16. It is very difficult to be sure of this however. I have counted them in the stages 2, 4, 5 (Fig. 28) and the impression always given is that 12 to 16 is the number.

In 3 (Fig. 28) the nuclear membrane is seen to be slightly irregular; the chromosomes have contracted towards the middle, and a blue network is seen surrounding them irregularly and is also seen between them. Then the nucleus begins to elongate in one direction and becomes oval-shaped. The chromosomes begin to take up a transverse position to form the equatorial plate, and at the same time the nuclear spindle appears, and the fact is strongly impressed upon one that it is formed out of the faint blue-stained network of the nucleus. This is supported by the fact that the nuclear membrane remains intact. In 5 (Fig. 28) the equatorial plate is seen and a well-formed spindle. The nuclear membrane becomes, at this stage, more indistinct and presents a granular appearance, which is probably the first indication of its

breaking down; but later than this I have not been able to follow the division. The number of nuclei contained in the oogonium after division is doubled, 143 and 218 having been counted.

It will be seen that although the general type of nuclear division conforms, to a greater or less extent, with that observed in the higher plants, there is one body concerned about which we are not quite so clear. The centrosphere which appears to be generally present both in animal and vegetable cells, has not been observed in the Fungi as satisfactorily as one would like. Observations have been made, however, which lead one to suspect that this is only due to the fact that our present methods of investigation are not sufficiently perfect to enable us to cope with the difficulties of observing structures, which even now cannot be observed without much careful manipulation in connexion with the very large nuclei to be found in the higher plants. It is probable that further investigation with improved methods of staining will confirm what has been already observed in connexion with the nuclei of the Hymenomycetes and Ascomycetes, and will demonstrate centrosomes in connexion with other nuclei also. With this exception I think we can safely say that there now remain only the Bacteria and Cyanophyceae in which a normal process of karyokinetic division of the nucleus has not been indicated.

The division which occurs in the oogonia and antheridia of *Cystopus* and *Peronospora* previously to fertilization is very interesting, and does not, so far as I am able to judge at present, admit of an explanation. The same phenomenon has been described by Eidam ('87) and Chmielewsky ('89) in *Basidiobolus*, one of the Entomophthoraceae, but not so far as I am aware in any other Fungus, except *Saprolegnia*, recently described by Trow ('95). In *Basidiobolus Ranarum* the vegetative cells contain only a single nucleus. In order to produce a zygospore, two cells lying together in the same filament put out a projection on each side of the common partition; the nucleus of each cell passes into the projection

thus formed, and each nucleus divides karyokinetically into two. The apical portions of these projections, each with a single nucleus, are then cut off by a partition-wall, and these two nuclei then degenerate and disappear. The partition-wall between the two contiguous cells is then resorbed, and the two masses of protoplasm, each with its nucleus, fuse together to form the zygospore. Chmielewsky ('89) was able to observe the ultimate fusion of the nuclei into one egg-nucleus. The fusion of the nuclei takes place very slowly, but in zygospores four weeks old they are always found fused together. The author makes the extremely interesting observation that quite ripe zygospores with fused nuclei would not germinate, but that unripe zygospores with the nuclei as yet not fused would germinate very easily when placed under suitable conditions. He explains the fact that the ripe uninucleate zygospores would not germinate because, obviously, they require a longer resting period.

This division of the sexual nuclei previously to fusion is exactly similar to what occurs in *Peronospora* and *Cystopus*, and according to Trow in *Saprolegnia* also. It would be interesting to know if it takes place in *Pythium*. The readiest explanation of the phenomenon would of course be that it is a reducing division, but this does not appear probable in the light of recent investigations on the reduction of the chromosomes in plants and animals, and also from the fact that, so far as I was able to observe in *Cystopus*, the number of chromosomes in the dividing nuclei in the oogonium is less than the number in the dividing nuclei of the fully formed oospore, as will be seen later. Moreover the explanation of this phenomenon, whatever it may be, is probably bound up with the explanation of the post-sexual process of reduction described by Klebahn ('88) in *Cosmarium* and *Closterium*, and by Chmielewsky in *Spirogyra*.

That 'we can only regard the nuclear divisions in oogonium and antheridium as phylogenetic reminiscences of the formation of gametes by cell division,' as suggested by Hartog ('91), is possible, but it seems to me that more facts are

required before a perfectly satisfactory explanation can be obtained.

Formation of Oil-drops.

Dangeard's observations ('90) are interesting and his figures of the appearances presented are correct as far as they go, but his interpretation is wrong. The central mass in his figures 6, 7, 8 is not an oil-globule, but the central, deeply stained, very dense mass of protoplasm already mentioned, containing a nucleus. The globules of oil at these stages are very numerous. In his figures 9, 10, 11, the central mass is undoubtedly oil. It is a pity that Dangeard took oospores at such very different stages to illustrate his general observations on the formation of oil. If he had carefully followed the gradual development of oil-drops, he would not probably have made the mistake of regarding the large central body found in the early stages of the development of the oospore as an oil-globule.

In sections cut from fresh specimens and from specimens soaked in corrosive sublimate and preserved in methylated spirit, small oil-drops in considerable numbers are to be seen soon after the separation of the oosphere from the periplasm. When the oosphere-wall has been distinctly formed, the oil-drops are very distinct.

These oil-drops gradually fuse together, and at certain stages one large oil-drop with a number of small ones is to be seen. In others, there are two large oil-drops and many small ones. The oil-drops are in cavities or vacuoles in the protoplasm and are much smaller than the vacuoles when they are of considerable size. This is due probably either to the contraction of the oil-drops or to the loss of a certain quantity of oil through soaking in spirit. The drops are coloured yellowish or blackish-brown in osmic acid solution.

All the oil-drops gradually fuse together into a somewhat irregular lumpy mass, and finally into the large central oil-sphere of the ripe oospore. At this stage, the large drop appears dense black after staining in osmic acid for some time.

The sections observed and described above were stained in osmic acid for twenty-four hours and mounted in dilute glycerine.

In the earlier stages, before the formation of the oosphere and just after its delimitation, a number of small, bright, refracting spheres were observed. These stained light yellow in osmic acid, and were probably small oil-globules in process of formation.

The antheridium is sometimes observed cut transversely in an oosphere. The dense protoplasm contained in it then nearly always presents the appearance of a crescent, owing to the protoplasm being more on one side of the tube than the other, and this is what Dangeard has probably mistaken for oil-crescents, and which I was inclined at first also to regard as due to the presence of oil.

Fertilization.

While the changes already described have been taking place in the oogonium, the nuclei of the antheridium have been undergoing division, and the number of nuclei at this stage is also now, so far as can be observed, about double the number at the beginning. The fertilizing tube has grown, and has at this stage pushed its way through the periplasm into the gonoplasm. A single nucleus and a small quantity of densely stained protoplasm passes from the antheridium into it down the side of the fertilizing tube (Fig. 11) to the apex (Figs. 12 and 13). The remainder of the tube is occupied by a thin lining layer of protoplasm and a large vacuole (Fig. 13).

The fertilizing tube now grows towards the centre of the young oosphere, around which a limiting membrane has not yet been formed. As it grows, the dense mass of protoplasm becomes reduced in amount, being used up probably to form the new growing wall. The apex of the fertilizing tube expands considerably, and it looks just as if this took place

in order to cover as much space as possible so as not to miss coming in contact with the nucleus of the oosphere (Fig. 14). In my preparations, the fertilizing tube was nearly always seen slightly contracted away from the protoplasmic layer immediately outside and in front of it, leaving a space. This was probably due to the contraction consequent on the addition of re-agents (Figs. 11, 13, 15 and 16). Occasionally, however, this contraction had not taken place (Figs. 12 and 14).

The fertilizing tube grows until it comes into contact with the central mass of dense protoplasm. This is shown in Figs. 15 and 16, two successive sections from the same oogonium; one showing the nucleus of the oosphere, the other the male nucleus in the fertilizing tube. The fertilizing tube has contracted slightly away from the protoplasm in its immediate neighbourhood, but its original position with respect to the nucleus of the oosphere can be distinctly seen. Unfortunately this oogonium was cut very obliquely, so that the antheridium itself could not be seen; nevertheless from examination of numerous sections at about this stage it is evident that the antheridium contains numerous nuclei. The apical part of the fertilizing tube is very thin and probably soft, and as soon as it has come into contact with the ovum-nucleus, the male nucleus is expelled and the tube immediately contracts, or rather collapses, and is withdrawn from the oosphere, leaving a large vacuole (*a*, Fig. 17) to mark its position. The two nuclei are left in close contact with one another (Fig. 17). The male nucleus is nearly always slightly smaller than the female. A delicate membrane, already indicated in Figs. 15 and 16, becomes now visible around the oosphere, separating it from the dense protoplasm. The two nuclei remain in contact for a short time and then fuse together (Fig. 20) to form the nucleus of the oospore.

It may be well to refer here to previous observations on the fertilization of the Peronosporeae. De Bary points out ('84) that in *Pythium*, nearly the whole of the contents of the

antheridium pass over into the oosphere; and if there is more than one antheridium, they all usually, but not always, empty their gonoplasm one after another into the oosphere.

Marshall Ward also ('83), in a very careful description of the process of fertilization in various species of *Pythium*, points out that 'the contents of the oogonium contract away from the wall towards the centre, strings and bands of protoplasm being left attached to the inner wall.' At a later stage, the central mass becomes rounded off, leaving a small quantity of protoplasm between it and the wall of the oogonium, the periplasm. This appears to be much smaller in amount than that found in *Peronospora* or *Cystopus*. The protoplasm of the antheridium, which contains a number of brightly refractive granules, sends out a fertilizing tube, which comes in contact with the oosphere or egg-cell. The contents of the antheridium then pass over almost entirely into the oosphere, a very small quantity only being left in the antheridium. A thin skin appears round the oospore and ultimately a thick envelope which is derived from the periplasm.

In *Phytophthora* again De Bary ('84) points out that the process of oospore formation is much the same as in *Pythium*, except that only a very small quantity of protoplasm passes over into the oosphere through the fertilization tube, and this portion is not distinctly separated beforehand. In *Peronospora* and *Cystopus* the passage of protoplasm could not be seen ('63–'84).

Neither Fisch ('85) nor Chmielewsky ('89) appear to have clearly observed the process of fertilization in *Cystopus*, and Fisch is not quite clear as to fertilization in *Pythium*.

It would of course be useless to offer at the present time any extended generalization from a comparison of the mode of fertilization of *Cystopus* as described in this paper with those methods described by earlier observers on other members of the Peronosporeae, as the latter are obviously incomplete; but it may be useful to point out that my observations on *Cystopus* are so far in general agreement with the observations on *Phytophthora* described by De Bary,

that the amount of protoplasm which passes over is very small; but do not agree with the observations made on *Pythium*, that nearly the whole of the protoplasm passes over. If it be found that the antheridium of *Pythium* contains numerous nuclei, and that these all pass over into the oosphere, as would appear to be clearly indicated by Marshall Ward and De Bary, and perhaps Fisch, it will afford an interesting comparison with *Cystopus*, and will probably lead to interesting results.

That fertilization takes place in the Saprolegniae as described by Trow, or as indicated by me for *Peronospora*, without the penetration of a fertilizing tube into the oosphere, would appear to be extremely doubtful in the light of these observations on *Cystopus*. If fertilization does take place in the manner described, then we have simply the passage of a male nucleus from the fertilizing tube through the protoplasm of the oosphere to the female nucleus without the intervention of a fertilizing tube, a state of affairs which is now known to take place, with the exception of a few little-investigated cases, only when antherozoids are formed. In all other cases, where the conditions under which the plant is grown do not allow the formation of antherozoids or motile spermatia, the male nucleus is carried right into the oosphere by a fertilizing tube: whilst in the archegoniate plants and in *Vaucheria*, a substance is excreted or formed which conducts the antherozoid down to the oosphere. Such evidence as this tends to show therefore that fertilization as is described in *Saprolegnia* by mere contact of a fertilizing tube with the oosphere, and subsequent passage of the male nucleus through it into the oosphere, does not take place as a rule.

Another interesting point to which it may be well to call attention is that the process of fertilization as here described does not differ in any essential particular from the process as it takes place in the Angiosperms, if we except the part played by the centrospheres, which I was not able to observe in *Cystopus*. In the Angiosperms, for example, according to

Guignard ('91) the extremity of the pollen-tube swells up on coming into contact with the embryo-sac, and pushes its way towards the nucleus of the oosphere. As soon as it comes near the latter the male nucleus passes rapidly through the membrane and comes into contact with the nucleus of the oosphere so quickly that it is rare to find it at any distance from the latter; a state of affairs which is almost exactly paralleled by what takes place in *Cystopus*.

In the Angiosperms the male nucleus, as it passes through the extremity of the swollen pollen-tube, is accompanied by a thin layer of protoplasm probably belonging to the generative cell. In *Cystopus* also the nucleus is accompanied by a small quantity of protoplasm at the time of fertilization, but whether or not any part of this passes with it into the oosphere could not be observed. In Angiosperms the male nucleus does not show any differentiated structure until it comes into contact with the female nucleus, when it swells up and assumes the normal structure of a resting nucleus. The two nuclei can be distinguished as separate structures up to the time of the formation of the chromatic segments. It is somewhat different in *Cystopus*; the male nucleus as it passes into the oosphere is certainly smaller than the female nucleus, but it appears to show much the same structure. It increases in size, and then fuses with the female nucleus; so far as I was able to observe the fusion is complete, and one nuclear membrane only can be seen at a stage much earlier than the appearance of the chromatic segments. These are however mere differences of detail, the important fact remains that the processes described are essentially similar in their chief features.

Maturation of the Oospore.

The fertilizing tube on its withdrawal from the oosphere is very much crumpled, as if by a sudden loss of turgidity due to the expulsion of its contents; but it soon regains to some

extent its original shape, although its apex appears in some sections to be disorganized (Fig. 18). In most cases a portion at least of the dense protoplasm which accompanied the male nucleus in its passage down the tube can be observed after the withdrawal of the latter.

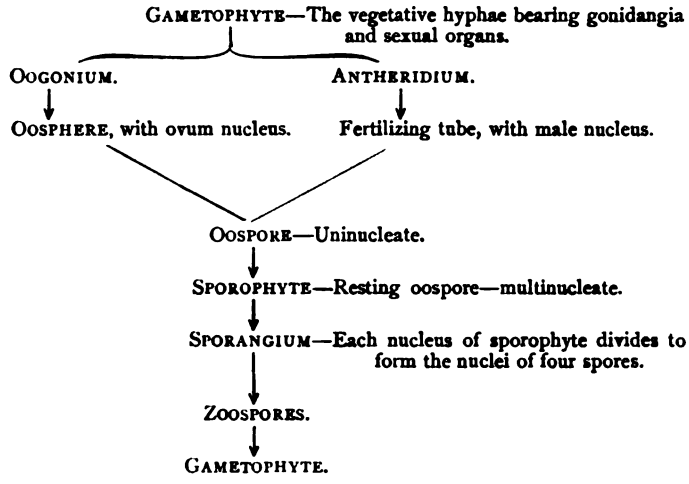
The fusion-nucleus in the oospore now begins to undergo changes. It increases in size; the network becomes more visible and stains more deeply, and the dense mass of protoplasm in its immediate neighbourhood begins to disappear, probably being used up to nourish the growing nucleus. The nucleolus then disappears, and the nuclear network divides up into chromosomes, which contract into an irregular spherical mass at the centre of the nucleus (Figs. 21, 22, 23), and presents the same appearance as occurs in the nuclei of the oogonium just previously to their division. The nucleus then divides, and although I have not been able to observe the details satisfactorily, it appears to follow the normal course of karyokinesis. The number of chromosomes present in the equatorial plate before division appears to be considerably in excess of the number observed in the nuclei of the oogonium, but it is very difficult to make sure. By counting as carefully as possible twenty to twenty-four or even more appear to be present, and the impression is produced that the number is certainly much larger than that observed in the oogonium.

Meanwhile the membrane around the oospore—the exospore—becomes distinctly visible as a moderately thick cell-wall. The columnar wall has appeared as minute projections on the inner layer (Figs. 21, 22), and further development of the wall accompanies the changes going on in the contents of the oospore, so that it is perfectly easy to follow the changes which take place in its further development in consecutive order, by using the formation of the wall as an index.

The two nuclei of the oospore (Figs. 24, 25) now undergo changes similar to those described for the primary nucleus, and finally each one divides into two, so that we have now

four nuclei in the oospore (Fig. 26). These are distributed irregularly in the protoplasm, which is very irregularly vacuolate, showing a large number of oil-vacuoles also. The four nuclei then divide again into eight, and successive divisions take place until thirty-two nuclei have been produced, at which stage the division stops. By the time this stage is reached, the oil-globules have united together into the single large central mass of oil, the protoplasm forming a layer around it in which the thirty-two nuclei are arranged in a single layer (Fig. 27). The oospore now appears to be mature, and the formation of the exospore and endospore to be complete. In the former three distinct layers can be seen; an inner comparatively thin homogeneous layer, a middle thick columnar layer, and an outer irregular warty layer. The endospore consists only of one very thick layer (Fig. 27). The oospore now enters upon its resting period, and it is interesting to note that it has already germinated to the extent of producing what we may regard as a multinucleate cell or sporophyte with thirty-two nuclei. I have never been able to observe a larger number of nuclei than this even in quite old oospores taken from very rotten material, so that it is probable that I am quite right in stating that this is its normal condition in the resting stage. Unfortunately I have never been able to observe the germination of the oospore, so that I cannot say at what period or under what conditions it begins to germinate; but De Bary ('63) has shown that in germination the whole of the cell becomes a zoosporangium; and, if his observation is correct, as is probably the case, that 100 or more zoospores are produced; and if we assume that each zoospore possesses one nucleus, as is also probable, then each of the thirty-two nuclei by dividing into four would give us 128 zoospores, a number sufficiently near De Bary's estimate to render it probably correct; we may therefore regard each of the thirty-two nuclei together with the protoplasm in connexion with it as the mother-cell of four zoospores, and the ripe oospore with thirty-two nuclei as the sporophyte. The life-

history of *Cystopus candidus* might then be represented by the following diagram.



LIST OF PAPERS QUOTED.

1807. PRÉVOST: Mémoire sur la Cause immédiate de la Carie ou Charbon des Blés &c., pp. 33-35. Montauban. (Abstracted in Ann. des Sci. Nat. Bot., Tome ii, 4th series, p. 155, by Tulasne.)

1847. TULASNE, L. R. and Ch.: Sur les Ustilaginées comparées aux Urédinées. Ann. des Sci. Nat. Bot. 3rd series, Vol. vii, p. 43.

1847. LÉVEILLÉ: Sur la Disposition méthodique des Urédinées. Ann. des Sci. Nat. Bot. 3rd series, Vol. viii, p. 369.

1848. BERKELEY: On the White Rust of Cabbages. Journal of the Hort. Soc. Lond., Vol. iii, p. 265.

1854. TULASNE, L. R.: Second Mémoire sur les Urédinées et les Ustilaginées. Ann. des Sci. Nat. Bot., Tome ii, 4th series, p. 108.

1859. HOFFMANN: Bot. Zeitung, p. 210.

1860. DE BARY: Sur la Formation de Zoospores chez quelques Champignons, premier mémoire. Ann. des Sci. Nat. Bot., Tome xiii, 4th series, p. 236.

1863. DE BARY: Recherches sur le Dévcloppement de quelques Champignons parasites. Ann. des Sci. Nat. Bot., Tome xx, 4th series, p. 14.

1872. CORNU: Monographie des Saprolegniées. Ann. des Sci. Nat. Bot., Tome xv, 5th series, p. 103.
1879. SCHMITZ: Untersuchungen über die Zellkerne der Thallophyten. Verhandlungen des naturhistorischen Vereines der preussischen Rheinlande und Westfalens. Bd. xxxvi, p. 345.
1882. BUSGEN: Die Entwicklung der Phycomycetensporangien. Dissertation; Sep. aus Pringsheim's Jahrbüchern für Wissenschaftliche Botanik. Bd. xiii, p. 22.
1883. MARSHALL WARD: Observations on the genus Pythium. Q. J. M. S., Vol. xxiii, new series.
1883. ZALEWSKI: Ueber Sporenabschnürung und Sporenabfallen bei den Pilzen. Dissertation; Flora, Bd. xli, p. 251, in the Separat., p. 9.
1883. ZALEWSKI: Zur Kenntniss der Gattung Cystopus. Bot. Centbl., Bd. xv, p. 215.
1883. SADEBECK: Untersuchungen über die Pilzgattung Exoascus &c., Jahr. der Wissenschaftlichen Anstalten zu Hamburg, p. 101. Figs. 17 and 20.
1884. DE BARY: Comp. Morp. and Bio. of the Fungi &c., Eng. Edit. 1887.
1884. STRASBURGER: Zur Entwickelungsgeschichte der Sporangien von *Trichia fallax*. Bot. Zeitung. 1884.
1885. FISCH: Ueber das Verhalten der Zellkerne in fusionirenden Pilzzellen. 58. Versammlung deutscher Naturforscher und Aerzte in Strasburg; see Bot. Centbl., Bd. xxiv, p. 221.
1885. FISCH: Bot. Zeitung, pp. 50–51.
1887. EIDAM: *Basidiobolus*, eine neue Gattung der Entomophthoraceen. Cohn's Beiträge zur Biologie der Pflanzen, Bd. iv, p. 181.
1888. KLEBAHN: Studien über Zygoten, Pringsheim's Jahrbücher, Bd. xxii.
1889. HARTOG: Recherches sur la Structure des Saprolegniées, Comptes-rendus de l'Académie des Seiences, Vol. cviii.
1889. HARTOG: Technique applicable à l'Étude des Saprolegniées, Bull. de la Soc. Bot. de France, Vol. xxxvi.
1889. CHMIELEWSKY: Zur Frage über die Copulation der Kerne beim Geschlechtsprocess der Pilze. Arbeiten d. neurussischen Naturf.-Gesellschaft. Bd. xiii, pp. 113–121. Odessa 1888 [Russisch]: See Bot. Centbl., Bd. xxxviii, p. 789.
1889. WAGER: On the Nuclei of *Peronospora parasitica* &c., Annals of Botany, Vol. iv, p. 127.
1890. DANGEARD: Recherches Histologiques sur les Champignons. Le Botaniste, 1890, p. 125.
1891. GUIGNARD: Nouvelles Études sur la Fécondation &c., Ann. des Sci. Nat. Bot., Tome xiv, 7th series.
1891. MANGIN: Sur la Désarticulation des Conidies chez les Péronosporées. Bull. de la Soc. Bot. de France, Tome xxxviii.
1891. HARTOG: Some Problems of Reproduction. Quar. Journ. Mic. Sci. 3rd series, Vol. xi.
1892. HUMPHREY: The Saprolegniaceae of the United States, with notes on other species. Trans. Amer. Philosophical Society, Vol. xvii, Part iii.
1892. ROSEN: Studien über die Kerne und die Membranbildung bei Myxomyceten und Pilzen. Cohn's Beiträge zur Biologie der Pflanzen, Bd. vi.

1892. WAGER: Observations on the Structure of *Cystopus candidus*. Brit. Ass. Reports, 1892, p. 777.
1893. GJURAŠIN: Ueber die Kerntheilung in den Schläuchen von *Peziza Vesiculosa*, Bull. Ber. d. Deut. Bot. Gesellschaft, 1893, p. 113.
1893. LISTER: Division of nuclei in the Mycetozoa. Jour. Linn. Soc. Bot. xxix, p. 529.
1893. PEGLION: Studio anatomico di alcune ipertrofie indotte dal *Cystopus candidus* in alcuni organi del *Raphanus Raphanistrum*. Estratto dalla Rivista di Patologia Vegetale, Vol. i, 1893.
1893. WAGER: On Nuclear Division in the Hymenomycetes. Annals of Botany, Vol. vii, 1893, p. 489.
1894. STRASBURGER: The Periodic Reduction of the number of the Chromosomes in the Life-History of Living Organisms. Annals of Botany, Vol. viii, 1894, p. 281.
1894. DANGEARD: Recherches sur la Reproduction Sexuelle des Champignons. Le Botaniste, 1894, p. 221.
1894. WAGER: On the presence of Centrospheres in Fungi. Annals of Botany, Vol. viii, 1894, p. 321.
1895. MACALLUM: On the Distribution of assimilated Iron Compounds, other than Haemoglobin and Haematin, in animal and vegetable cells. Q. J. M. S., Vol. xxxviii, new series, 1895.
1895. ISATSCHENKO: Zur Histologie der *Pholiota Aurea*, Fr. SB. Naturf.-Gesell. in St. Petersburg, am 15. (27.) Februar 1895. See Bot. Centralbl. lxiii, 1895, p. 103.
1895. ISTVÁNFFI: Ueber die Rolle der Zellkerne bei der Entwicklung der Pilze. Ber. d. Deut. Bot. Gesell. 1895, Bd. xiii, p. 456.
1895. HARTOG: On the Cytology of the Vegetative and Reproductive Organs of the Saprolegnieae. Trans. of the Royal Irish Acad., Vol. xxx, p. 649, 1895.
1895. OLTMANNS: Ueber die Entwicklung der Sexualorgane bei *Vaucheria*. Flora, p. 388, 1895.
1895. TROW: The Karyology of *Saprolegnia*. Annals of Botany, Vol. ix, 1895, p. 609.
1895. HARPER: Beitrag zur Kenntniss der Kerntheilung und Sporenbildung im Ascus. Ber. d. Deut. Bot. Gesell., Bd. xiii, 1895, p. 67.
1895. DANGEARD: Mémoire sur la Reproduction Sexuelle des Basidiomycètes. Le Botaniste, 1895, p. 119.
1896. WAGER: Reproduction and Fertilization in *Cystopus candidus*. Annals of Botany, Vol. x, 1896, p. 89.

EXPLANATION OF FIGURES IN PLATES XV AND XVI.

Illustrating Mr. H. Wager's paper on *Cystopus candidus*.

All the figures have been drawn with the aid of the camera lucida, and the apochromatic 2·0 mm. apert. 1·40 of Zeiss with ocular 8 (× 1000), except Fig. 28 which was drawn with ocular 18 (× 2250), and Fig. 3 drawn with ocular 12 (× 1500).

Fig. 1. Small piece of mycelium showing nuclei and haustoria.

Fig. 2. Mycelium with sporangiophores containing nuclei, previous to the formation of zoosporangia. Fig. 2 *a* is a sporangium.

Fig. 3. Sporangiophore with seven nuclei and three more passing in from the hypha. Oc. 12.

Fig. 4. Young oogonium just forming; the irregular shape of the nuclei clearly seen and the lines produced by the streaming in of the protoplasm at the base.

Fig. 5. Young oogonium soon after its delimitation from the mycelium. An antheridium is seen attached to one side and the accumulation of a denser protoplasm is to be seen commencing in the oogonium on the side near the antheridium. In this oogonium there were 115 nuclei altogether, and about six in the antheridium.

Fig. 6. A later stage than Fig. 5. The nuclear network is distinctly visible, and the germinal papilla projecting from the oogonium towards the antheridium.

Fig. 7. Portion of section of an oogonium at a later stage than Fig. 6. The nuclei have much increased in size and the nuclear network is very clear.

Fig. 8. Section of an oogonium in which the protoplasm has begun to contract towards the centre. The nuclei show their chromosomes contracted in the centre joint preliminary to division. Thick strands of protoplasm are seen connecting the central mass of protoplasm to the wall of the oogonium.

Fig. 9. Later stage than Fig. 8. Nearly all the nuclei have divided, and are collecting at the periphery of the central mass. A deeply stained dense mass of protoplasm is seen in the centre, in contact with which is the ovum nucleus, produced by the division of one of the original nuclei of the oogonium.

Fig. 10. An antheridium attached to the wall of an oogonium, just beginning to push out its fertilizing tube. A dense mass of protoplasm and several nuclei are seen in and near the projection. This dense mass of protoplasm stains deeply in a similar manner to that in the centre of the oogonium or young oosphere.

Fig. 11. Further stage of development of an antheridial fertilizing tube. The antheridium now contains a large number of nuclei produced by the division of those originally present, but the fertilizing tube contains only one nucleus, together with a mass of deeply stained protoplasm; these are passing down the sides of the tube towards the apex.

Fig. 12. A fertilizing tube at a slightly later stage than preceding, showing the male nucleus with protoplasm at the apex.

Fig. 13. Oogonium showing ovum-nucleus with its deeply stained mass of protoplasm, and a fertilizing tube with nucleus at the apex. Owing to the action of re-agents the fertilizing tube is shown slightly contracted away from the protoplasm into which it is pressing. The central mass of protoplasm which will become the oosphere shows, as well as in previous sections, a very characteristic foam-structure.

Fig. 14. Later stage than Fig. 13. The apical wall of the fertilizing tube is now becoming very thin, and the tube itself is expanding at the apex into a bladder-like structure. The wall of oosphere has not yet begun to form.

Figs. 15 and 16. Two successive sections of the same oogonium, showing the fertilizing tube in contact with the central or ovum nucleus.

Fig. 15. Ovum nucleus shown, and fertilizing tube which has contracted away from it, probably owing to the action of re-agents.

Fig. 16. Shows the apex of the fertilizing tube with nucleus, the deeply stained mass of protoplasm in contact with the ovum nucleus is shown on the wall of the space produced by the contraction of the fertilizing tube. These two sections taken together show that the male nucleus is brought quite into contact with the ovum nucleus by means of the fertilizing tube.

Fig. 17. Section showing the male and female nuclei in close contact with one another. The male nucleus is expelled from the fertilizing tube in some way, probably by a gradual thinning and ultimate rupturing of its apex, the fertilizing tube being immediately withdrawn, a large vacuole (*a*) being left to indicate the place where it was. The two nuclei in the egg are still surrounded by a dense mass of protoplasm. The wall of the oosphere can now be seen.

Fig. 18. Portion of a section of an oogonium immediately after fertilization, showing the fertilizing tube withdrawn to the outside of the young oosphere, and presenting an appearance as if ruptured or disorganized at the apex.

Fig. 19. An older oogonium with young oosphere showing the empty fertilizing tube on the outside of the latter. The antheridium contains still a small quantity of protoplasm, and a number of small degenerating nuclei.

Fig. 20. Section of an oogonium at a later stage than Fig. 17, showing the nucleus of the oospore shortly after the fusion of the male and female nuclei. Two nucleoli can be seen, and the nucleus is of an oval shape and still surrounded by the dense mass of protoplasm. The wall of the oosphere, the exospore, is now distinctly visible.

Fig. 21. Oospore nucleus at a later stage than in Fig. 20. It contains a considerable number of granules, probably chromosomes, and a very distinct nuclear membrane. It is surrounded by a small quantity of the dense deeply stained protoplasm only. As the latter disappears the nucleus takes a more intense stain and increases in size. The wall of the oospore just begins to show the appearance of the columnar layer of the exospore.

Fig. 22. Oogonium with oospore, slightly later stage. The columnar layer of the exospore is now visible as a number of minute projections on the inner wall. The chromosomes of the nucleus are contracting towards the centre, probably preparatory to division.

Fig. 23. A still later stage of oospore formation. The columnar layer is more strongly developed. The endospore has not yet begun to develop. The chromosomes are still more contracted towards the centre. An antheridium attached to the wall of the oogonium shows a number of small degenerating nuclei. The periplasm

is still more or less granular, but the nuclei are not so clearly visible in it; they are probably in process of degeneration.

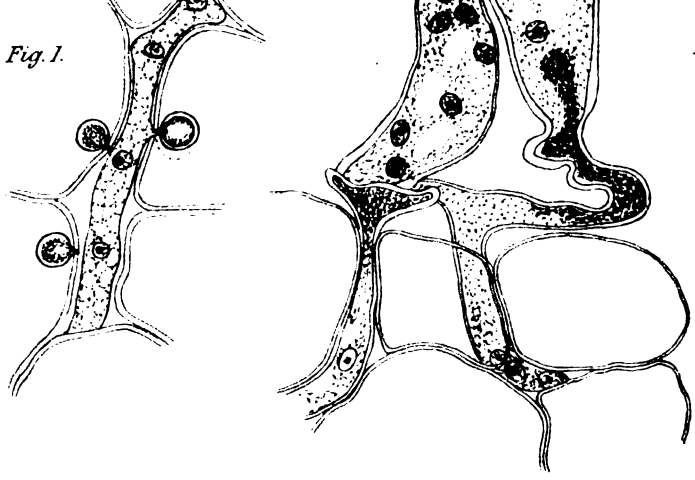
Fig. 24. Portion of section of an oogonium showing the oospore with two nuclei. Each nucleus possesses a distinct network and a small nucleolus. The protoplasm is losing its characteristic foam-structure and is becoming more irregularly vacuolate. Some of the vacuoles indicate oil-spaces.

Fig. 25. Portion of an oogonium with contained oospore, showing two nuclei just after division. This oospore contained four nuclei, produced by the division of two nuclei similar to those in Fig. 24. The columnar layer of the exospore is more highly developed. Spaces in which oil was contained are shown.

Fig. 26. Oospore with four nuclei. The endospore is just beginning to form, as a thin layer just inside the innermost layer of the exospore. The columnar layer has apparently been completed and the third is now being formed. The whole or nearly the whole of the periplasm has been thrown down upon the wall of the spore in the form of a dense homogeneous irregular layer, only here and there is to be seen any connexion with the wall of the oogonium.

Fig. 27. Ripe oospore in section, showing seven nuclei in the parietal layer of protoplasm. The section is taken from an oospore containing thirty-two nuclei. Each nucleus with its protoplasm may be regarded as a mother cell of four zoospores. The central space shown is filled, in the fresh oospore, with oil. The endospore is very thick. The fertilizing tube of the antheridium is also shown surrounded by an exospore.

Fig. 28. Five stages in the division of the nuclei of the oogonium. Four and five show the spindle-figure. It will be seen that the nuclear membrane persists at least until the formation of the equatorial plate and probably later.



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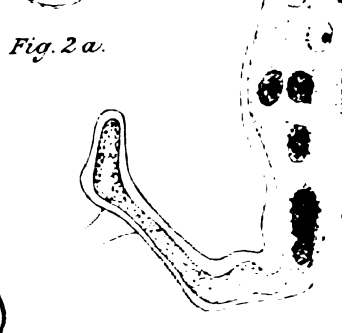
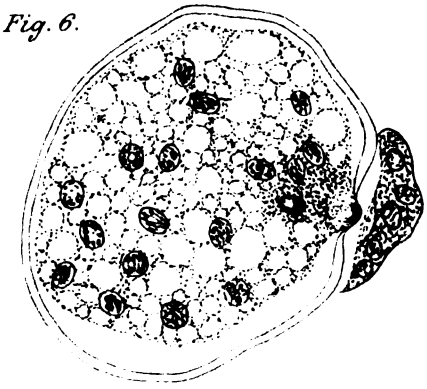


Fig. 2 a.

Fig. 3



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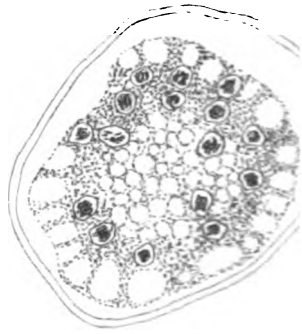

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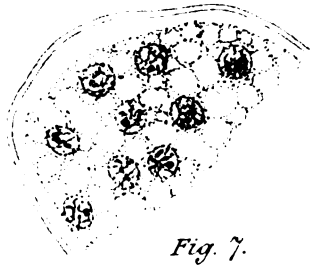

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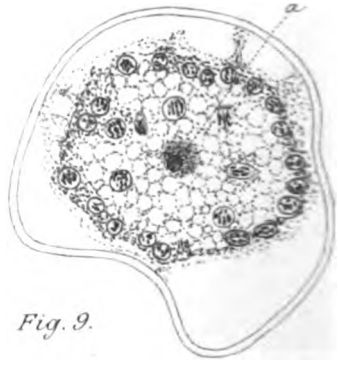

Fig. 9.

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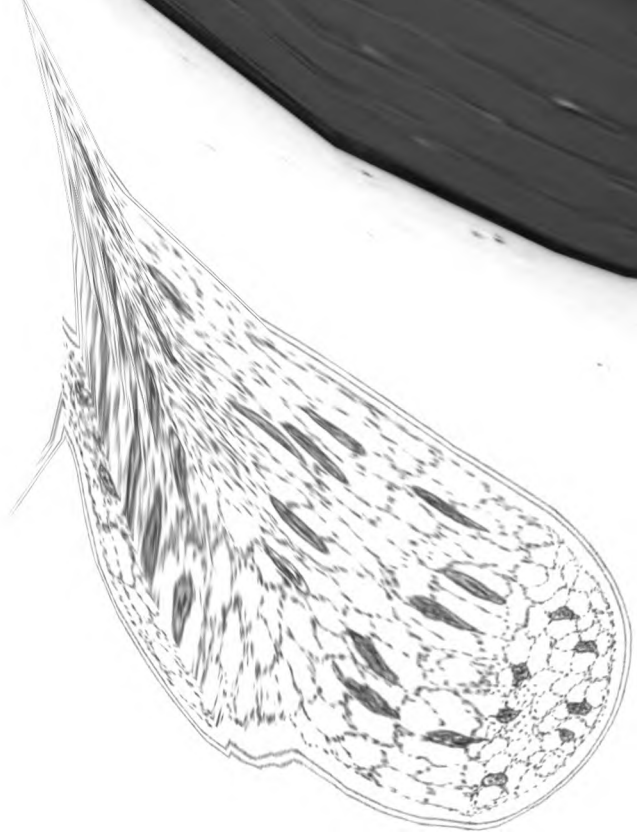

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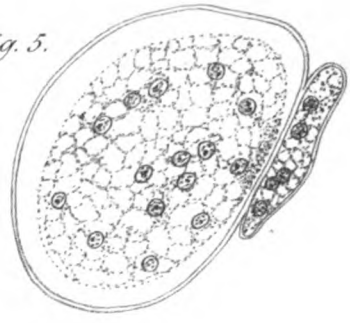
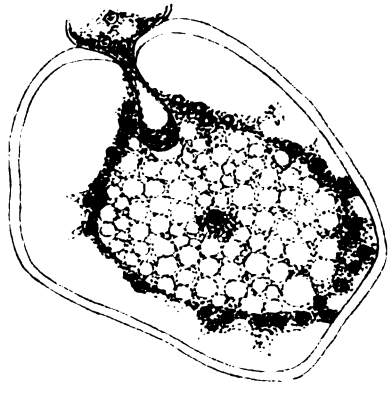


Fig. 10.

Fig. 13.



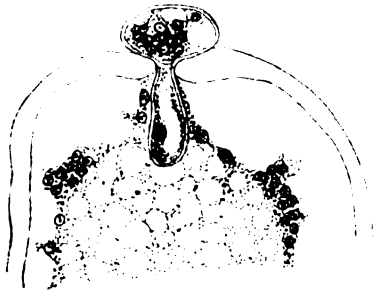


Fig. 11.

Fig. 14.

Fig. 12.

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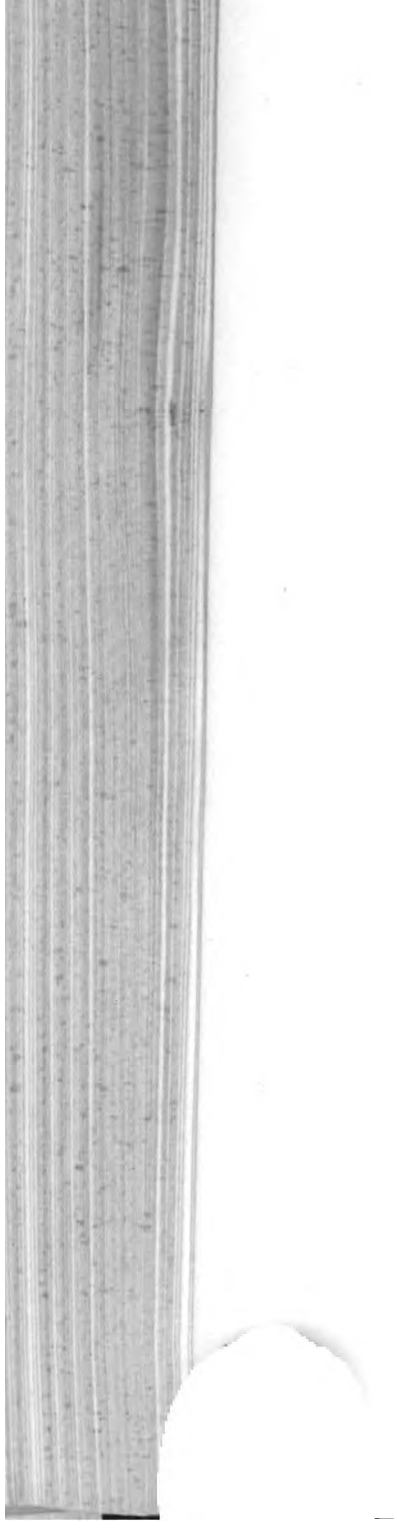
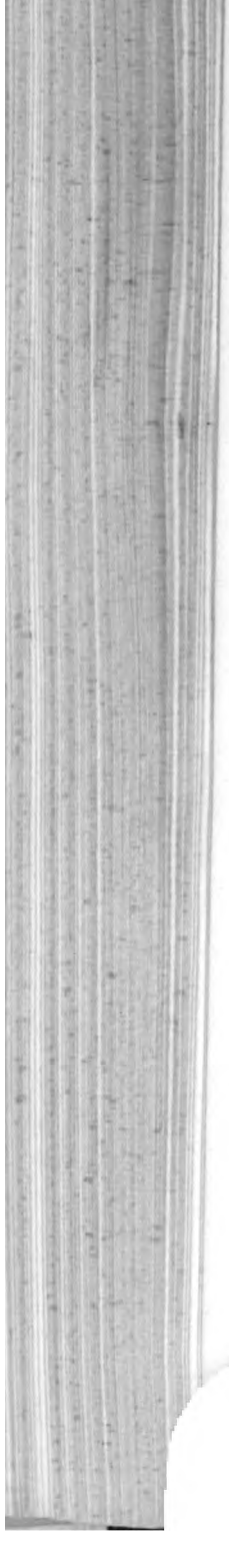
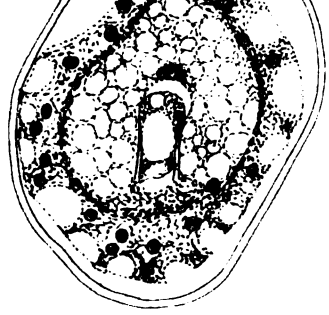






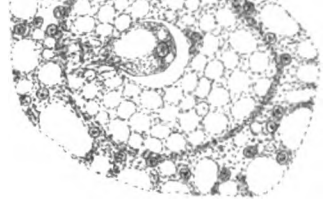


Fig. 22.

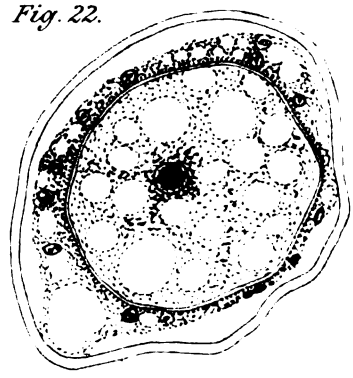


Fig. 20.

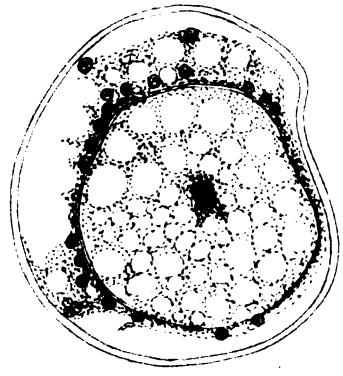


Fig. 23.

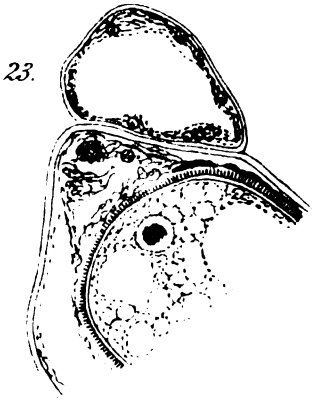


Fig. 21.

H. Wager del.

WAGER.— CYSTOPUS.

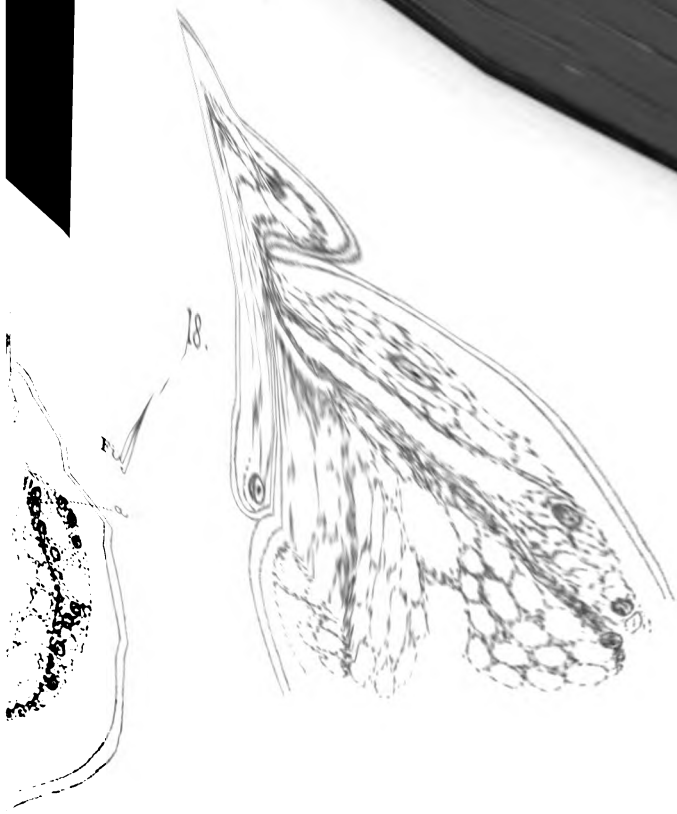


Fig. 19.

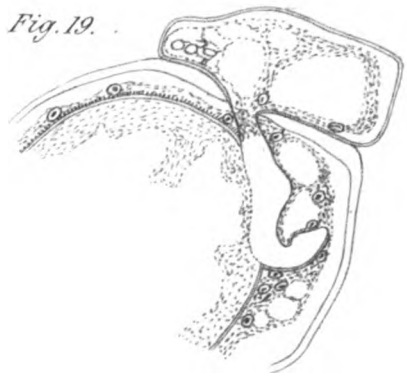


Fig. 25.

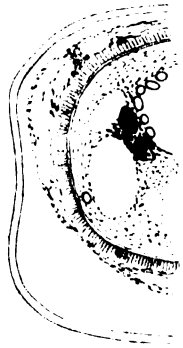


Fig. 26.

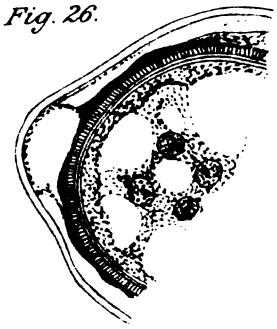


Fig. 27.

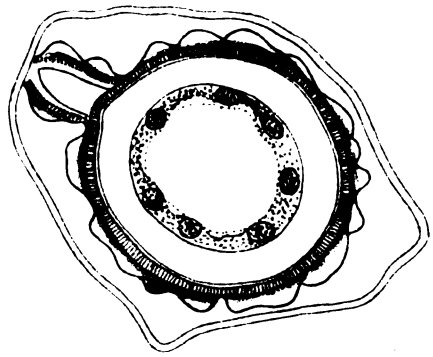
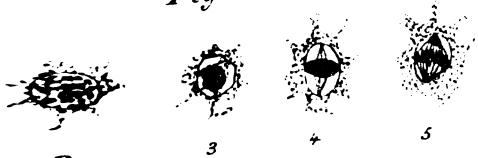


Fig. 28.



The Development of Mutinus caninus (Huds.), Fr.[1]

BY

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With Plates XVII and XVIII.

IT is my purpose to trace in the present paper the course of development of *Mutinus caninus*, as shown in a closely connected series of the so-called eggs. Three objects are kept in view :—

1. To determine from the course of development in the youngest stages the relationship, if any, between the Phalleae and the Clathreae—the two principal sections of the Phalloideae.

2. To subject the development of the wall of the receptaculum—the latter consisting of merely a stipe in this species—to a more critical examination than it has hitherto received.

3. To make suitable record, by means of the illustrations, of the most important typical stages of development which could be selected from forty-four fructifications of this species which have been examined, and of which I have permanent preparations.

Although a valuable contribution to our knowledge of the

[1] Contribution from the Cryptogamic Laboratory of Harvard University, No. XXXVI. Prepared under the direction of Dr. W. G. Farlow.

[Annals of Botany, Vol. X. No. XXXIX. September, 1896.]

Phalloideae has been recently made by A. Möller[1], still, up to the present time, only two of the Phalleae have been described and figured in their youngest stages, on account of the great difficulty of procuring such stages. Both investigations were made by Ed. Fischer. The first of these, published in 1890, was on *Ithyphallus impudicus*[2], and the second, in 1895, on *Mutinus caninus*[3]. In both these investigations, however, the supply of material was deficient in important young stages. In the case of *I. impudicus*, this resulted in an erroneous view of the course of development at a point of great importance in determining the relationship between the Phalleae and Clathreae. A more correct view is presented in the recent paper on *M. caninus*, yet here also the lack of closely connected stages compels the author to make the reservation that the course of development outlined in the earlier paper may, after all, be the true one, thus leaving the question still open. But these points, and that in regard to the origin of the gleba, will be treated more explicitly in the following pages.

The material used in my investigation was collected in Somerville, Mass., in July, 1894. In this connexion I desire to express my thanks to Mr. B. M. Duggar and to Mrs. H. A. Ricker for directing me to a locality where this plant—which, in our region, is usually found growing solitary or in clusters of but few individuals—was growing in great luxuriance and abundance in a neglected area.

Through the kindness of Prof. Farlow this material has been compared with authentic specimens of *Corynites Ravenelii*, B. and C., in the Curtis Herbarium, and it is undoubtedly of the same species. There does not seem to be good ground for regarding *C. Ravenelii*, B. and C., as specifically distinct from *Mutinus caninus* (Huds.), Fr.

[1] Brasilische Pilzblumen. Jena, 1895.

[2] Untersuchungen zur vergleichenden Entwicklungsgeschichte und Systematik der Phalloideen. Denkschr. d. Schweiz. Naturf.-Ges., Bd. XXXII, 1, 1890, p. 22.

[3] Die Entwickelung der Fruchtkörper von *Mutinus caninus* (Huds.). Berichte der Deutschen Bot. Ges., 1895. Bd. XIII, p. 128.

METHODS.

In general, alcohol-material as needed for study was stained in the mass for twenty-four hours in P. Mayer's paracarmine—a 70 per cent. alcoholic stain. This penetrated well. After washing and dehydrating, the fructifications were finally brought into pure cedar-oil. After they had sunk to the bottom in this, paraffin was gradually added to saturation. The fructifications were then transferred to the paraffin-bath, and finally imbedded in paraffin melting at 58° C.

The sections were usually cut $6\frac{2}{3}$ μ thick, with a Minot-microtome, and were attached to the slide with Mayer's albumen-fixative after floating out on the slide in water. The paraffin was removed with xylol. The sections were run down through the grades of alcohol to water, and were then stained on the slide with aqueous solution of safranin for about five minutes.

They were mounted temporarily in water. All the drawings showing hyphal structure were made from preparations in water. They were then permanently mounted in glycerine by running under the cover-glass a mixture of two volumes of concentrated glycerine and one volume of distilled water. After allowing this to concentrate by evaporation from under the edges of the cover-glass, the mounts were cleaned and then sealed with hot glycerine jelly and finished with Bell's cement.

Attempts were made to find staining methods which would give still better results than the one described, but they were unsuccessful in general. With very small fructifications not having the gelatinous layer of the volva differentiated, haematoxylin stains could be used, and the dark colour which they gave to the walls was a great help in the camera lucida work of such medium power drawings as my Figs. 1 and 2. A slight staining of the walls of the hyphae with Ehrlich's haematoxylin, just before using the safranin, may be made with advantage in some cases. The method of employing potassium hydrate with thin unattached sections is described in another place.

Final stage.

The main features of *M. caninus*, and the chief terms to be employed, may be brought to mind by a brief description of the more familiar mature stage. In this stage a fusiform-cylindrical stipe issues vertically upward through the ruptured apex of a fleshy bag, called the volva or peridium. The apex of the volva is usually near the surface of the ground. The white or reddish stipe is hollow, about 6 to 15 cm. long by 1 cm. in diameter, and has a brittle wall of chambered structure (Figs. 13 and 14). The partition-walls of these chambers consist of a modified hyphal tissue called pseudoparenchyma (*k*, Fig. 15). In the lower part of the stipe the chambers open outward, giving a perforated outer surface.

At the time of elongation of the stipe the upper one-sixth to one-third of its length is covered by a closely adnate, greenish spore-mass called the gleba, which has in some degree the fetid odour characteristic of the Phalloideae. Deliquescence of the gleba, accompanied by the formation of sugars[1], at once sets in, and the gleba soon drips away or is removed by flies and other insects[2], thus exposing the spore-bearing (i. e. gleba-supporting) part of the stipe. This portion is more highly flesh-coloured than the portion below, and the chambers of its wall open inwards into the main central cavity of the stipe.

The volva consists of three layers: a thin outer layer continuous with the cortical layer of the mycelial strand; a thin inner layer—the inner layer of the peridium—in the plane in which the volva splits away from the gleba at maturity; and a broad middle layer, highly gelatinous in nature, and hence called the gelatinous layer of the volva.

[1] Rathay und Haas: Ueber *Phallus impudicus* (L.) und einige Coprinus-Arten. Sitzungsb. d. Mathem.-Naturwiss. Akad. zu Wien, 1883, Bd. LXXXVII, 1. Abth. p. 18.

[2] Rathay und Haas, l. c.; Fulton, T. W.: Dispersion of Spores of Fungi by Insects. Annals of Botany, Vol. iii, 1889, p. 207; Gerard, W. R., In Bulletin of Torrey Botanical Club, Vol. vii, 1880, p. 30.

The fructification, or compound gonidiophore, in the stages before elongation of its stipe has occurred, is commonly called an 'egg.' The eggs usually arise at the ends of fine lateral branches of the mycelial strands, but they are occasionally found sessile laterally on the strands.

YOUNG STAGES.

First egg.—The youngest body that I have been able to recognize from its microscopic structure as an egg, and not the mere vegetative tip of a mycelial strand, is shown in median longitudinal section in Fig. 1. It was a soft, hyphal body having a length of $\frac{2}{5}$ mm. and a transverse diameter of $\frac{1}{5}$ mm. The mycelial strand bearing it had a diameter of 40 μ. A bundle of about half a dozen hyphae *M*, Fig. 1, extends from the medullary portion of the mycelial strand up through the middle of the section into its upper portion. There the bundle branches, forming a dense, sheaf-like head *N* of interlacing and anastomosing hyphal branches very difficult to follow. The rest of the section is a more open structure *C*, consisting of loosely interlacing hyphae curving and branching and crossing in all directions. At the base of the egg, hyphae from the cortical portion of the mycelial strand may be followed up into this tissue *C*, where they branch, curve about, have the characteristic form of the tissue, and soon become lost by leaving the plane of the section. One such hypha is indicated by *e*.

Several eggs in this stage of development have been met with in preparing a complete series of stages. Rarely a hypha from the medullary bundle *M* will send out a branch into the cortical portion *C*, but here it immediately takes on the characters of this portion, and curves in and out among the other hyphae, branching as they do. In the mycelial strand such branches occasionally pass from the medullary into the cortical portion and become a part of it.

Contrary to the idea that has prevailed, these three parts of the egg—*C*, the continuation in the egg of the cortical

system of the mycelial strand; *M*, the continuation of the medullary system of that strand; and *N*, the upper end of *M*—come to play very unequal parts in later development. Of these parts *C* becomes the outermost layer of the volva; *M*, the bundle of medullary tissue connecting the mycelial strand with the base of the stipe; while the sheaf-like head *N* gives rise to all the remaining parts, including stipe, gleba, and gelatinous layer of the volva.

In eggs of this stage stained *in toto* in Delafield's haematoxylin or in P. Mayer's haemalum, the hyphae of the medullary bundle were about 4 μ in diameter. They had very thin walls and were divided by extremely thin cross-walls into cells about 20 μ long. In most of these cells there could be seen a deeply-stained, opaque, spherical mass about 2 μ in diameter—presumably the nucleus. In the sheaf-like head *N*, the tissue was so intricate from the crossing of hyphae in different planes that the cross-septa could not be made out with certainty. The nuclei were, however, very numerous in this portion and not more than 3 or 4 μ apart. The hyphae of the cortical portion are from 4 to 5 μ in diameter and from 20 to 25 μ long.

Ed. Fischer[1] described as young a stage of *Ithyphallus impudicus*, shown in his Fig. 18, which differs from my Fig. 1 chiefly in the greater number of hyphal branches from the medullary bundle (*M* of my Fig. 1) passing out nearly at right angles into the cortical layer. In having more such branches in the cortical layer of its egg than exist in *M. caninus*, the cortical tissue of the egg *I. impudicus* merely shares a character of the cortical system of its mycelial strand, as the latter has been made known by De Bary[2]. On account of the presence of such branches in the egg of *I. impudicus*, Fischer separated the tissue which I have designated by *C* into an outer peripheral zone and an inner mass bordering on the medullary bundle. The inner mass he supposed to be identical with the

[1] L. c., 1890, p. 22.

[2] Zur Morphologie der Phalloideen. In Beiträge z. Morph. u. Physiol. der Pilze. Abhandl. d. Senkenb. Naturf.-Ges., Bd. V, 1864, p. 204.

intermediate tissue (*Zwischengeflecht*) of later stages. In reality the mass in question and the peripheral zone become only the outermost layer of the volva; hence a distinction between the two ceases to be of importance.

Second egg.—The next stage in the differentiation of the sheaf-like head *N* is shown in Fig. 2, with the same magnification as in the preceding figure. The egg of this stage had a length of about 1 mm. and a transverse diameter of ½ mm. The general arrangement of parts is the same as in Fig. 1. The cortical tissue *C* is continued up from the mycelial strand and forms the outer portion of the egg. The bundle of medullary tissue *M* has about the same position as in Fig. 1, and can be traced as a bundle up to about the centre of the egg, where it branches as in Fig. 1, and becomes lost in the spherical mass *N*, lying in, and almost wholly constituting, the upper half of the egg.

The peripheral portions at the upper end and sides of the head *N* have pushed outward against the cortical layer *C*, and have become differentiated into a more loosely interwoven structure *L*, in which many of the hyphae have a radial position. Many of the hyphae along the inner surface of the cortical layer *C*, pass into, and take part in, the formation of the marginal portion of this more open structure; still the general appearance of the section is such as to indicate that the structure is, on the whole, of medullary nature, arising from *N*. In this stage, two regions may therefore be indicated in the head *N*; the more open portion *L*, just described; and the dense portion *O*, consisting of hyphae intricately interwoven and anastomosing, and with their nuclei at only very short distances apart.

At *m*, Fig. 2, the medullary bundle *M* breaks up and passes into the sheaf-like head *N*. One of the few hyphae of that bundle has chanced to lie in the plane of the section for a portion of its length after leaving the bundle. This hypha is designated by *m*. It passes obliquely upward towards the left, and certainly at two points gives off branches into the open portion *L*, and is also connected on the other side with

the dense portion *O*, by anastomosing branches or processes. There is great significance in the direct connexion of the medullary bundle *M* with the outer portion of the sheaf-like head at its base; for it shows an original direct connexion of the medullary bundle with the base of all parts of the later plant except the outermost layer of the volva.

Third egg.—The egg of this stage is larger, measuring 1½ mm. long by ⅘ mm. wide. By far the greater part of the increase in growth has been in the sheaf-like part, which now measured 700 μ in diameter. Differentiation of the dense portion *O* has now given rise to an axial column of interlacing hyphae, extending upward from the upper end *m* of the medullary bundle of Figs. 1 and 2 (*R*, Fig. 3). In the preparation this column is about 500 μ long by 100 μ wide; it has taken an orange-red stain, and is thus quite distinct from the surrounding tissue on its sides; a longitudinal direction slightly predominates in the position of its hyphae. This column *R* persists as an axial column of gelatinous tissue until the period of elongation of the stipe.

Lying against the column *R*, on its sides and upper end, is the remainder of the dense tissue *O*, which is now—especially in its upper portion—a denser structure than *R*. It is now the region where the nuclei are most numerous and nearest together in the hyphae. The hyphae of this portion still retain their connexion with the column *R*; they are densely interwoven and many of them are arranged radially.

A gelatinous accumulation has set in at *G*, in the open tissue *L*. It is the beginning of the gelatinous layer of the volva. This gelatinous substance is regarded as a transformation-product of the hyphal wall [1]. It lies close against the dense portion *O*, in a narrow zone of irregular width—ranging from 20 to 80 μ in this preparation. It is stained orange by the safranin employed.

[1] See De Bary, Beiträge z. Morphologie u. Physiologie der Pilze, I. Reihe, (1864), p. 195; and also Van Bambeke, Sur la Morphologie du *Phallus impudicus* (L)., Bull. de la Société Royale de Botanique de Belgique, T. XXVIII, 1889, p. 39.

The features of this stage consist in the beginning of—

1. The differentiation of the main central column *R* of the stipe, and

2. The gelatinous accumulation in the layer of that nature in the volva.

Fourth egg.—In the more advanced stage of Fig. 4, the central column *R* is more distinctly set off from the tissue at its sides. Near the column this tissue has become looser (the portion *A*), but still remains dense and with many nuclei in its dome- or bell-shaped peripheral portion. At its upper end, the column *R* has connexion with this dense portion, thus marking out the permanent connexion that exists at the apex of the receptaculum between the column *R* and the tissues of the gleba and gelatinous layer of the volva in Fig. 5, and in those of later stages. In the early stage of Fig. 4 the column is not contracted at this place of connexion, as in Fig. 5, but its hyphae spread apart, forming a mass with an angle of divergence of about 60° between its opposite sides.

This stage shows a great increase in the gelatinous accumulation, which now fills all the loose structure *L*, in the upper part of the egg, and is extending downward in this structure, but keeping close against the cortical layer *C* in its downward course.

Fifth egg.—This stage is intermediate between that of Figs. 4 and 5. The central column spreads out less at its upper end and has thus become cylindrical in form. With the growth of the egg, the dense zone has become still further removed from the column at the sides. Along the inner surface of this zone, differentiation of the hymenium is in progress; this is shown under low magnification by the presence of a dark, narrow, interrupted line lying parallel to the dense zone, and evidently formed from an inner portion of it. Under high magnification this line is found to consist of swollen portions of hyphae and of their branches arranged side by side.

Intermediate Stages.

It is convenient to consider as *young* stages all those in which there is not yet any well-marked differentiation of the wall of the receptaculum; as *intermediate* stages, those in which the wall of the receptaculum is present but not yet crowded into folds; and as *advanced* stages those with the walls of the receptaculum folded.

Sixth egg.—The rudiment of each part of the mature plant is now recognizable, occupying the position which it has in the old egg. This is apparent by a glance at Figs. 10 and 5. The latter, Fig. 5, represents a median longitudinal section through an egg about 2 mm. in diameter, drawn under lower magnification than the preceding figures. Comparing this with the general features of those figures, we observe, that the vigorous growth in the sheaf-like head *N*, which has gone on with its rapid differentiation, has greatly changed the relative proportions and apparent positions of the parts of those stages; that the cortical layer *C* forms the outer covering of the egg, and is still present in greatest amount at its lower end; and that the medullary bundle *M* runs upward through this basal mass to the lower end of the central column *R*. With the exception of the cortical layer *C*, and the medullary bundle *M*, all the rest of the egg has been found to arise from the sheaf-like head *N*.

Examining the latter structure more closely, it may be seen that the central column *R* is now slightly contracted at its apex, and is taking on the slightly fusiform outline characteristic of the cavity of the stipe in the mature plant. Just outside the column *R* the rudiment of the wall of the stipe *S* is becoming differentiated from the loose tissue *A*. Following Ed. Fischer, this loose tissue *A* will be called *intermediate tissue* (*Zwischengeflecht*), but this term should not be used for the Clathreae.

In later stages, splitting away of the volva from the gleba and receptaculum occurs through the zone *i* in a plane parallel with the outer surface of the egg. On the peripheral side of

i there is a broad mass of gelatinous tissue, horse-shoe shaped in section; it is the gelatinous layer of the volva, and has already been described as making its way downward in the loose tissue *L* and keeping close to the cortical layer. Now it quite encloses on the top and sides the central mass of tissue, in which important developmental changes are in progress. Although the question must be left unanswered, it may be asked if the function of this gelatinous layer is not protective?

On the opposite side of *i*—at *H*—the young basidia of the hymenium are forming. A cluster of these basidia from near the point indicated by the end of the line from *H* is shown in Fig. 6. As nearly as I could determine, the hyphae which give rise to the basidia are composed of short cells, the cross-septa of which do not show very distinctly, however, in my preparations. From each of these cells an outgrowth occurs which may form one of the swollen basidial cells, or may become septate, like the hypha from which it arises, and then give rise to a cluster of basidia. The latter is more likely to occur when the outgrowth originates at some distance from the palisade-like layer, in which the young basidia stand side by side. The tramal connexions of the basidia are towards the dense zone *i* and the apex of the column *R*.

In Fig. 6, the intermediate tissue *A*, lying between the hymenial layer *H* and the stipe, has been separated from the tramal tissue through the formation of the basidia. In Fig. 5, the tramal tissue and intermediate tissue are in connexion at several points where basidia have not yet formed. In the preceding stage intermediate portions of the hyphae were swollen with protoplasm in the region where the hymenium was developing. The conditions there, taken in connexion with those of the present and with those of later stages to be described, seem to indicate that the ready separation from, and pushing away of, the intermediate tissue *A* by the forming basidia may be favoured by atrophy of the hyphal connexions with that tissue. In a slightly later stage the separation becomes more complete (Fig. 8).

In his recent paper on *M. caninus*, Ed. Fischer has pointed out the connexion between the masses of tramal and intermediate tissue, and has concluded that the gleba must originate from the differentiation of portions of the intermediate tissue[1]. The course of development does not favour this conclusion (see Fig. 4), but shows rather that the intermediate tissue *A* and that of the gleba, although both arising from the sheaf-like head *N*, by the differentiation of originally intimately connected portions, tend to almost wholly lose such connexions through developmental changes, and have nothing further in common in their development. In such further development the glebal tissue, making its tramal connexion with the upper end of the central column *R* of apparently increasing functional importance, takes on the formation of spores; while the intermediate tissue *A*, losing also its connexions with the sides of the column *R*, but retaining those with the basal portions of the egg, goes on to form a receptaculum of varied form for the different genera—a supporting and elevating structure for the spore-mass.

The rudiment of the wall of the stipe is in the stage of Fig. 5, a narrow layer, *S*, of swollen portions of hyphae very irregular in form and very intimately connected with the loose intermediate tissue *A*, of which they form a part. The hyphae of this rudiment lie close against, and often interlock with, the longitudinally running hyphae of the column *R*. The details of this structure are shown in Fig. 7, which represents under higher magnification a portion of the rudiment of the stipe with adjacent tissues at the lower end of the stipe. The exact position of this area in Fig. 5 is indicated by *S*. The hyphae on the left in Fig. 7 belong to the tissue of the central column *R*; those on the right are in the intermediate tissue *A*; the more deeply shaded hyphae between constitute the rudiment of the stipe *S*. The septa of the hyphae did not show distinctly in the preparation, and no attempt at their representation has been made in the figure. The hyphae of

[1] L. c., 1895, p. 136.

this rudiment run in very irregular and zigzag courses in all directions, and give out short lateral processes or branches, but these are probably in part anastomosing connexions which have been cut in sectioning. These hyphae stain deeply. Higher up the stipe the hyphae of the stipe and those of the column *R* are less interlocked than in this figure.

Relationship of Phalleae and Clathreae.

We may here consider the bearing of the facts of early development upon the views held as to the relationship between the Phalleae and Clathreae. As a stage of development has now been reached which is about the same as that illustrated by Ed. Fischer for *I. impudicus*[1], in his Figs. 20 and 21, it becomes possible to compare the actual course of development with the ideas that are prevalent. It should be understood, however, that Fischer's statement of the course of development was advanced tentatively, until a fuller series of stages should be available for study, and that in his late paper on *M. caninus*[2] he outlines and favours in some important features the course of development which I have presented here, but which I had already worked out independently in my inaugural thesis, before the publication of Fischer's paper. While advancing such newer views, he also states his inability to determine from his preparations whether his earlier scheme of development might not be the true one after all.

In Fischer's earlier account the central column of the intermediate stage was regarded as identical with the medullary bundle *M* of my Fig. 1, although Fischer was not sure that a portion of the upper end of the column might not be formed from a part of the sheaf-like head of *M*. The gelatinous layer *G*, the dense portion *i*, and the tissue of the gleba, were regarded as arising from the sheaf-like head *N*. By a vigorous interstitial growth, and the production of radially arranged

[1] L. c., 1890, p. 22.

[2] L. c., 1895, p. 130.

hyphae, the sides of the head would be carried farther and farther apart, until finally they would become directed obliquely downward about the sides of the medullary bundle *M*. *N* would thus become a dome resting on the top of the bundle *M*, with its sides extending obliquely downward about that bundle. Throughout this process the point *m* of the youngest stage would be regarded as close underneath and against the closed end of the forming dome, and having in later stages the position of the point *r* in my Fig. 5. The basidia were regarded as arising on the lower surface of the dome from the portions of *N*, which were so recurved as to face towards *M*.

This theory was directly misleading as regards the phylogenetic relationships of the Phalleae. In the Clathreae the hymenial tissue is within the receptaculum, and the free ends of the basidia face towards the periphery; in the Phalleae the hymenial tissue is on the outside of the receptaculum, and the free ends of the basidia face towards the longitudinal axis of the plant, as shown in Figs. 5 and 6. In these two respects the conditions in the Phalleae are exactly the reverse of what they are in the Clathreae. These differences, taken in connexion with the course of development that was accepted, would be reconciled by an origin of the Phalleae from some Anthurus-like member of the Clathreae by a protrusion upward—from the upper end of the rudiment of the receptaculum of that ancestor—of the tissue which was to give rise to the gelatinous layer of the volva and to the hymenium. By spreading out above the receptaculum and then recurving downward about it on the outside, the hymenial structure would have come to have the position on the outside of the receptaculum characteristic of the Phalleae. This would also have given the relative inversion in the direction of the basidia. The basidia, having a position in the young Clathreae close against the inner side of the receptaculum, with their free ends directed toward the periphery of the egg, would, upon coming outside the receptaculum and recurving downward, come to face in the opposite direction, or toward the axis. If

such had been the origin of the Phalleae, there ought to have been intermediate forms left somewhere on the way, but no such form could be found.

The actual course of development has proved to be very different from that presumed. The original medullary bundle *M* does not become the central column *R*; but instead merely connects the lower end of the latter with the mycelial strand. The rudiment of the stipe does not arise from the primitive cortical tissue lying next to the medullary bundle *M*, but from a secondary tissue differentiated in the medullary sheaf-like head *N*. Instead of being originally only connected with the mycelial strand in a roundabout way through the tissue of the central column *R*, there was originally a more direct connexion with the tissue giving rise to the gleba and the gelatinous layer *G* at the lower end of the future stipe—at the point *m*, Figs. 4 and 5. That this primitive condition should become lost, and that an apparently secondary connexion by way of the upper end of the column *R* through anastomosis of hyphae should become of more permanent functional importance, is exceedingly interesting, but no more surprising than the well-known later developmental change, through which the originally free surface of the hymenial or glebal structure becomes so firmly adnate to the receptaculum as to permit the gleba to be torn from its tramal connexions on the opposite side and carried aloft by the elongation of the receptaculum.

Since the original connexion of the tissue giving rise to the gelatinous layer of the volva, to the dense zone *i*, and to the hymenium, was directly connected with the point *m*, the outline first presented as to how the Phalleae might have arisen from the Clathreae becomes plainly contradicted by the facts, which show differences so fundamental as to point rather to independent origins for these two sub-families.

Seventh egg.—This egg was about 4 mm. long by 3 mm. in transverse diameter. On account of the curvature of the parts in the egg, my sections are not median for the full length of the receptaculum. The portion of the figure below

the line *S*, Fig. 8, had to be drawn from another section in the same series. The outer portions of the section have been omitted in the figure.

The gleba and its relations to the intermediate tissue *A* are of especial interest. The separation of the gleba from the intermediate tissue *A*, in progress in Fig. 5, is now more complete, and has resulted in the formation of a cavernous space, shown in Fig. 8. Some feeble hyphal connexions still remain between the projecting lobes of the gleba *H* and the tissue *A*. In some of the furrows of the former structure, hyphae are seen in a position suggesting their having been pulled away from the tissue *A*, in the movements which brought the portions of the hymenial surface to which they were attached to the bottom of the furrows. The formation of the cavernous space may be due to a very vigorous growth of the dome-shaped gelatinous layer of the volva, which, by becoming a portion of the shell of a sphere of greater diameter, would tend to pull tissues attached to it laterally away from the central axillary core of tissue more firmly attached at top and bottom.

The formation of the folds of the hymenial surface was stated by Ed. Fischer, in his study of *Ithyphallus tenuis*, to be due to the repeated formation of new basidia, which crowd their way to the hymenial surface, pushing in between basidia already lying in that surface[1]. The steps leading to such an increase in the hymenial surface are shown in Fig. 6.

The rudiment of the wall of the stipe is thicker and denser than in the preceding stage. A portion of the rudiment from another egg in about the same stage of development as that of Fig. 8 is shown in Fig. 9. Ed. Fischer has found in *I. tenuis*[2] and in *I. impudicus*[3], a loose tissue with numerous spaces between its hyphae, forming a clear zone between the

[1] Ed. Fischer, Zur Entwicklungsgeschichte der Fruchtkörper einiger Phalloideen. Annales du Jardin Botanique de Buitenzorg, Vol. vi, 1887, p. 10.

[2] L. c. 1887, p. 8, fig. 8.

[3] L. c., 1890, p. 25, fig. 22.

central column *R* and the rudiment of the wall *S*. Such a condition does not generally exist; for in *M. caninus* the rudiment of the stipe *S* and the central column *R* are in close contact (Figs. 9 and 11).

The hyphae of the rudiment *S*, Fig. 9, are richer in protoplasm than they are in other portions of the intermediate tissue. This protoplasm does not form a continuous mass distributed uniformly throughout the length of each hypha, but is broken up into short masses, as shown in the figure. It is possible that this appearance of the hyphae is due to their being cross-septate into short cells, but I could not be sure of this.

Eighth egg.—In this more advanced intermediate stage (Fig. 10), the egg has become elongated—measuring 9 by 5 mm.—and its parts have undergone important changes. By the repeated formation of folds and the growing together of such folds where they came into contact, the gleba has become a structure of innumerable closed or labyrinthine chambers lined by the hymenium, the basidia of which now bear spores. There is no longer a cavity between the gleba and the intermediate tissue *A*. Where the lobes of the former have pressed against the latter, the tramal tissue has grown out against the tissue *A*. It is by such growth that the gleba becomes adnate to the stipe in the older stages.

The stipe has become more fusiform, and a chambered structure is beginning to form in its wall. Under a magnification of sixty diameters, the future chambers show as light areas marked out from the more deeply stained portions which become their walls. Under higher magnification (Fig. 11), the transition from the light areas *c*, *c* to the darker portions is so gradual that one cannot fix upon any point at which the light portion *c* ends and the darker part begins. The tissue composing both the future chambers and their walls was so dense, and the lateral inflations of the hyphae were so irregular in form, as to make it quite impossible in a camera-lucida drawing to follow the hyphae accurately in sections attached to the slide and mounted in

water or glycerine, even though such sections were only 6½ μ thick. It is, however, possible to treat such thin sections so as to render this practicable.

The steps of the process are as follows:—

1. Do not attach the section to the slide.
2. Remove the paraffin.
3. Carefully run down through the alcohols to water.
4. Treat for about five minutes with 7% solution of potassium hydrate.
5. Wash out the potassium hydrate with water.
6. Stain with aqueous solution of safranin or with Hoffman's blue.
7. Crush out somewhat by careful pressure on the cover glass.

The process is rather long, and very delicate manipulation is required; but in successful preparations the dense tissue opens sufficiently so that it may be drawn, and the hyphae separate from each other slightly so that they may be followed in their course with certainty. Fig. 11 has been drawn from such a preparation. It shows the same intricately interwoven structure that we have seen in the preceding stages of the rudiment of the stipe. This holds true also for the portion of the rudiment lying next to the central column *R*. Ed. Fischer[1] has, however, repeatedly figured and described this portion in quite as early a stage as consisting of swollen hyphal ends extending outwards from the central column *R* at right angles to that column, and forming a continuous palisade-layer along its entire length. I have made a very thorough search in early intermediate stages for such a palisade-layer, but fail to find in the region below the spore-bearing part any structure next to *R* that can be so regarded upon close examination. The arrangement of the protoplasm in the hyphae in block-like masses—which are short cells, perhaps—greatly increases the difficulty of determining the

[1] L. c., 1887, figs. 13 and 17; l. c., 1890, figs. 16 and 24; Neue Untersuch. z. vergleich. Entwicklungsgeschichte u. Systematik d. Phalloideen. Denkschr. d. Schweiz. Naturf.-Ges., Bd. XXXIII, 1, 1893, figs. 61, 62, 72; l. c., 1895, p. 131.

real course of these crowded hyphae in ordinary preparations. Their real courses and arrangement in the region in question have, however, been carefully traced in Figs. 9 and 11.

Hyphae may be seen (Fig. 11) passing through the light areas *c*, *c* and showing in their different portions the variations in intensity of stain characteristic of the adjacent tissue. I have traced a hypha from one light area through the darker partition-wall into the next light area. The points marked *c*, *c* in the figure are not the only ones at which crossing of interwoven hyphae occurs, but these marked points are places in which further advancing differentiation ceases, and in which a formation of gelatinous substance sets in. Into these lightly staining areas, channels of a similar nature may lead from the outer surface of the stipe, and thus bring the intermediate tissue *A* into intimate communication with a much greater surface of the pseudoparenchyma of the wall (Fig. 14).

I have stated that the hyphae of the dark partition-walls may extend into, or may issue from, the areas *c*, *c*. They may arise directly from the intermediate tissue *A* and be traced for considerable distances in the dark walls, crossing and becoming intertangled with the other hyphae of those walls. In these darker portions between the light areas *c*, *c*, and between these areas and the central column *R* on the one side, and the tissue *A* on the other, rapid growth and further differentiation into pseudoparenchyma occurs in later stages. The inflations of the hyphae become greater, and short branches are formed. The development of pseudoparenchyma is most active along the surfaces where the partition-walls are in contact with the gelatinous tissue of the future chambers *c*, *c*, with that of the column *R*, or with the intermediate tissue *A*. As a result of such growth, the partition-walls become more and more compact along their surfaces and more open in structure further in (Figs. 14 and 15).

Hyphal knots.—Ed. Fischer[1] accounts for the wall of the

[1] L. c., 1887, pp. 17 and 18.

receptaculum, and for the dense shell of pseudoparenchyma about each chamber, and next to the central column *R*, and next to the intermediate tissue, by stating—

1. That in early development the tissue of the rudiment of the stipe differentiates into a series of isolated dense hyphal knots, which are separated from the central column *R*, from each other, and from the tissue *A*, by narrow open spaces;

2. That an outgrowth of swollen hyphal ends—pseudoparenchyma—then takes place into these spaces from the isolated knots of hyphae, from the central column *R*, and from the tissue *A*;

3. That these approaching plates of pseudoparenchyma become loosely grown together later;

4. That the central portions of the hyphal knots become constantly looser and more gelatinous, finally forming the chamber-cavities of the wall.

There are important features in the above that need modification. It seems probable that Fischer's hyphal knots are the portions *c*, *c*, Fig. 11, which stain lighter in my preparations; but these portions are certainly not isolated from each other by open spaces as he states and figures[1]. On the contrary, the light-staining portions are closely connected together from their first appearance by a densely interwoven tissue of which they are only portions. These connexions are retained in the various preparations which I have seen. It is quite true that branches from the tissue of the light portions—the chambers later—crowd their way into the plates and become pseudoparenchyma, thus increasing the area of these plates: pseudoparenchymatous hyphae, favourably situated in the partition-wall, also branch and help to augment the same surfaces.

Nature of the 'pits' in the 'spore-bearing' part of the wall.—In the mature plant pits are found opening from the wall of the stipe into its main central cavity. These pits are in the upper portion of the stipe—in its spore-bearing portion.

[1] L. c., 1887, figs. 13, 17, 18.

They are shown in section in Figs. 10 and 13. The identity of such pits with chambers in the lower part of the wall has recently been pointed out by A. Möller in the case of a Brazilian Mutinus[1]. After stating that the formation of the chambers in the wall of the stipe is from below upward, and hence that they may be more or less incompletely formed at the top of the stipe, he adds further that the deep pits in the wall opening into the central cavity near its upper end must be regarded as chambers lacking a wall on the side next to the main central cavity.

Before reading Möller's work I had reached the same conclusion in regard to the pits of *M. caninus*, but through different evidence. That these pits are of the same nature as the chambers lower down in the stipe, and that they differ from such chambers merely in the absence of a wall on the side towards the cavity of the stipe, becomes very probable when one splits a mature plant lengthwise, and views the chambers and pits from the side of the main central cavity of the stipe. Near the upper end, deep pits, somewhat hexagonal in cross-section, occur; towards the lower end, the chambers are found, each covered by a thin membranous wall, which is so puffed out as to readily show that the form of the chamber is like that of the pit. The change from chambers to pits occurs rather abruptly, yet between the two regions there is a narrow zone within which it is possible to find all intermediate conditions between the two extremes. In some cases the membrane almost clóses the chamber, but thins out towards the middle, leaving a minute opening into the chamber-cavity. In other cases the membrane does not cover more than half of the side of the pit, and in others it is a mere border about the pit. Fig. 13 shows some of these intermediate stages in section, but they are best seen by looking against the inner surface of the stipe.

The mode of origin of the pits may be seen by examination of suitable sections from an egg in the stage of Fig. 10. Fig. 12 shows a section through one of the pits in that stage.

[1] L. c., 1895, p. 93.

In passing up the wall, the band of pseudoparenchymatous tissue between the light portions *c*, *c* and the central column *R* (Fig. 11) becomes narrower, until finally the light portion is in contact with the central column. At points where the light portions are merely tangent to the central column, deeply stained pseudoparenchymatous hyphae may be found running upward from below in the zone next to the column, then crossing through the surface of contact and passing into the dark zone above. The portion of the hypha lying in the light area shows the colour characters of that area. Treatment of the sections by the potash method already described is advisable.

It is unusual to find hyphae which can be traced for as long a distance as is necessary in crossing the pore, but many hyphae may be found which pass from the intermediate tissue *A* through the deeply staining portions—where they show the irregular lateral inflations of pseudoparenchymatous hyphae—and then into the light area *c* (Fig. 11). Of course the greater number of hyphae in a thin section can be followed for only a portion of such a course, when they pass out from the plane of the section. The relations of the intermediate tissue *A* to that of the light portion *c* of a pit are exhibited in Fig. 12, which is a section from an egg in a slightly different stage of development from that of Fig. 11. This light portion was in the upper part of the intermediate zone, and would probably have formed a pit with a narrow membrane about its mouth. Scattered hyphae may be seen passing into *c*, Fig. 12, from the column *R*, but I have been unable to trace them on into the partition-wall *k*. The general connexion of the tissue of the pit *c* with the tissue *A* shows that it should be classed with that tissue—as are the chambers lower down the stipe—rather than with the column *R*. In older stages, when the growth of the pseudoparenchymatous walls increases the size of the other chambers, it also increases the capacity of these chambers or pits, and numerous hyphae from the central column *R* may be seen passing into them and occupying all available space.

Ninth egg.—The following stages in the development of the egg are better known, and can be treated more briefly. A section through the stipe and adjoining parts of an egg 13 mm. long is shown in Fig. 13. The section is median at the upper and lower ends of the stipe, but is slightly oblique about midway between the ends on account of the curvature of the stipe. This gives a somewhat irregular outline to the wall on its inner side in that region.

In its lower portion, the wall of the stipe contains but a single layer of chambers, and these have thin walls; near the middle, the wall is thicker, and its chambers are more numerous and more irregular in form; higher up, the pits have become swollen with tissue from the column *R*, and the wall has become very massive. This massive portion is not thrown into folds in the advanced stages of the eggs. It is the portion upon which the gleba rests. Can it have become massive in order to give a more rigid support for the gleba in its development?

Some chambers in the wall of the stipe are closed, their tissue having no connexion with the intermediate tissue *A* except through pseudoparenchyma. A portion of the wall, showing many places of direct connexion of the intermediate tissue with that of the chambers, is shown in Fig. 14 under low magnification. Such passages into the chambers become the pores or perforations which are found in the outer surface of the stipe after its elongation. The figure shows also by the darker shading the denser structure of the wall of pseudoparenchyma along the surfaces. Perhaps the more favourable conditions for the development of that tissue in those surfaces may be due to proximity to a tissue containing available food.

Mode of thickening the wall in the spore-bearing part[1].—In the upper part of the wall of the stipe in Fig. 8, the pseudoparenchymatous hyphae form a single narrow zone bounding the pits, and so forming a very irregular outline for that

[1] Compare also Ed. Fischer on this topic in his already cited paper of 1895, p. 135, figs. 5–8.

part of the stipe. The two opposite sides of this zone are parallel, as shown in Fig. 12. The undifferentiated tissue *A* is thus separated from the tissue *c* of the pits by only a single layer or plate of pseudoparenchymatous nature, and extends in the opposite direction to the gleba. Ed. Fischer has described such a condition of this part of the wall and of the tissue *A* as the permanent condition in *Mutinus Mülleri* [1].

Such a condition is not permanent, however, in *M. caninus*. The two surfaces of the wall in this region are no longer parallel in the older stage of Fig. 13. The section of the outer surface has become nearly a straight line. This has resulted from the formation of a second plate of pseudoparenchyma just outside the first one. It has not conformed to the first plate, but extends more directly from prominence to prominence of that plate. The hyphae of the tissue *A*, which are shown in Fig. 12 directly connected with the first plate *k*, and those in the angles between the pits became pseudoparenchymatous also when the second plate was formed, and may be seen connecting the two plates in Fig. 15. I desire to call attention to this point, for although the two plates of the partition-wall between the rest of the tissue *A* and the pits *c* are not formed simultaneously, in other respects the mode of formation of this partition-wall is the same as that which I have described in connexion with the chambers lower down the stipe.

The differentiation of the second plate began at the outer surface of the wall in the region below the pits, and then advanced upwards; hence this plate cannot be regarded as homologous with the veil or pileus of other genera of the Phalleae.

Advanced Stages.

As already stated, the advanced stages may be regarded as those in which the walls have become folded. In these stages the cell-like bodies of pseudoparenchyma, which constitute

[1] L. c., 1890, p. 32, fig. 25.

the walls of the chambers, increase greatly in size, and it becomes difficult to conceive of their having originated by lateral inflation of hyphae. The growth of the walls is more rapid than that of the tissue of the chambers, and, as a result, the even-surfaced chamber-walls of Fig. 11 are thrown into a series of folds like those of a Chinese lantern, to use the old illustration. As the growth in length of the stipe after the folding of the chamber-walls still continues to be more rapid than the growth in length of the egg, the wall of the stipe as a whole becomes thrown into a series of folds in this species.

In these changes the intermediate tissue *A* and the tissue of the chambers become torn, the latter tissue and that of the central column become more highly gelatinous, and the gleba splits away from the volva in the plane of the inner layer of the latter. During this last change, the volva is burst open at its apex by the receptaculum, which gradually pushes its way up through the opening by the straightening out of its folded walls. It is now believed that the straightening out of the folded walls and consequent elongation of the stipe is due to changes in the form of the cells situated at the inner and outer surfaces of each angle of each fold. At the inner surface of the angle, the cells are wedge-shaped as though by compression; on the outer surface, they are elongated and flattened as though by a pull longitudinally. Both of these sets of cells become more spherical in form during elongation of the stipe. Such a change in the form of the cells at the inner surface of the angle must tend to lengthen that surface and so straighten out the fold; the change in form of the cells on the outer surface of the angle must tend to shorten that surface and so help straighten the fold. The changes in form of the cells on both surfaces of each cell therefore work together in straightening the fold. In Errera's paper taking up this subject, the change in form of the cells at the angles of the folds is regarded as due to a true process of growth[1].

[1] L. Errera, Sur le glycogène chez les Basidiomycetes. Mémoires de l'Acad. roy. de Belgique, t. XXXVII, (1885), p. 52 of reprint.

Ed. Fischer's paper of two years later treats the process as one of turgescence [1].

SUMMARY.

In its earliest recognizable stage, the egg consists of the cortical and medullary tissues of the mycelial strand, continued directly upward from the strand. Of these tissues, the medullary bundle spreads out at its upper end and forms a dense sheaf-like head by repeated branching and anastomosing.

The cortical layer of tissue becomes the outer wall of the volva; the sheaf-like head gradually differentiates into all the other parts of the older egg.

In such differentiation the central column *R* is the first to be set off, arising from the axial tissue of the head and from below upward.

Formation of the gelatinous layer *G* of the volva now begins in the periphery of the head. The dense portion between the peripheral zone and the central column *R* becomes looser in its inner and lower parts about the column, there forming the intermediate tissue *A*, but remains dense in its upper and outer portions, forming a compact dome-shaped mass. In these changes the direct connexions from the original medullary bundle *N* to the dense dome-shaped mass and to the lateral portions of the sheaf-like head disappear, while indirect connexions by way of the central column *R* are retained, and become of apparently great functional importance.

Along the inner surface of the dense zone and next to the intermediate tissue *A*, the rudiment of the gleba *H* arises from the clustered swollen ends of lateral branches of the tramal tissue. These hyphal ends take position in a palisade-layer facing the intermediate tissue. By the crowding in

[1] Bemerkungen über den Streckungsvorgang des Phalloideenreceptaculums. Mittheil. d. Naturf.-Ges. in Bern, 1887, p. 142.

of new hyphal ends (basidia) into this layer, its surface becomes greatly enlarged and the layer is thrown into folds. During this process it becomes torn from the intermediate tissue.

The rudiment of the stipe arises in the intermediate tissue *A*, lying next to the central column *R*, by the formation of a zone of deeply staining tissue rich in protoplasm.

Somewhat later, masses of tissue in the dense and intricately interwoven rudiment of the stipe show a tendency towards gelatinization. These masses mark the position of the later chamber-cavities in the wall. Towards the upper end of the stipe, such masses are in contact with the central column *R*, and they mark the position of the 'pits' which open into the main central cavity of the stipe in mature stages of *M. caninus*.

The tissue of the rudiment of the partition-walls, situated about and between the gelatinous masses, continues its development into pseudoparenchyma, which, with conditions for its development most favourable where it is in contact with the gelatinous tissues and the presumably food-supplying tissue *A*, becomes relatively more and more dense next to those surfaces, and more open, by contrast, between those surfaces. In later stages, before folding of the wall has gone too far, this gives to the partition-wall the appearance of being composed of two plates rather loosely connected together.

At no stage in the course of development was the rudiment of the stipe found to consist of hyphal knots isolated from each other and from the surrounding tissues by narrow open spaces.

The chamber-walls are thrown into folds through a more rapid growth of the pseudoparenchyma than that of other parts of the egg.

Final elongation of the stipe and elevation of the gleba is brought about through the straightening out of the folds in the chamber-walls.

General Conclusions.

Although the receptaculum and the gleba are both differentiated from the early sheaf-like head of medullary tissue, still the pseudoparenchymatous walls of the former seem to have nothing more immediately in common with the hymenium either in origin or in mode of formation. There is no good reason for considering the two structures homologous.

The steps by which the lower and lateral portions of the sheaf-like head—those portions bearing the future hymenium —lose their original direct connexion with the medullary bundle *M* and become split away, as it were, from the stipe from below, have no parallel in the Clathreae, and remind one rather of the changes that occur in the formation of the pileus in some of the Agaricineae.

Medullary tissue gives rise to both the tissue of the chambers of the wall and to the pseudoparenchyma of its partition-walls, and one portion of a hypha may undergo the modification characteristic of a chamber, while another portion differentiates into pseudoparenchyma. In the Clathreae, as pointed out in my paper on *Anthurus borealis* [1], cortical tissue continued upward from the mycelial strand forms the pseudoparenchyma, while the tissue of the chambers is of medullary origin, and not connected with that of the partition-walls.

Such fundamental differences must signify that the Phalleae and the Clathreae are not directly related; although they may properly be regarded as two parallel series of forms, on account of their general features of resemblance in spore characters and external form.

This investigation was carried on in the Cryptogamic Laboratory of Harvard University in the winter of 1894–95, under the direction of Professor W. G. Farlow. I desire to express my sincere thanks to Professor Farlow, and also to Professor Thaxter, for helpful suggestion and criticism received in the

[1] A North American *Anthurus*— its Structure and Development. Memoirs of the Boston Society of Natural History, Vol. iii, No. XIV, 1894.

course of the investigation, and for the use of papers which would otherwise have been inaccessible to me. My thanks are also due to Mr. H. M. Richards and to Mr. H. L. Jones, Assistants in the Botanical Laboratories, for numerous favours which have facilitated my work.

EXPLANATION OF FIGURES IN PLATES XVII AND XVIII.

Illustrating Dr. Burt's paper on the development of *Mutinus caninus* (Huds.), Fr.

Note.—All figures were drawn with an Abbé camera-lucida.

Lettering common to all the figures.

M, medullary bundle of young stages; *N*, sheaf-like head of *M*; *C*, cortical layer; *G*, gelatinous layer of the volva; *Gl.*, gleba; *H*, hymenium; *L*, outer and more open portion of *N*; *O*, dense portion of *N*; *R*, central column; *S*, stipe; *c*, tissue of chambers and pits; *i*, region of innermost layer of volva; *k*, partition-wall of pseudoparenchyma; *m*, point at which sheaf-like head *N* arises from medullary bundle *M*.

Figs. 1–4. Median longitudinal sections through eggs in successive young stages, showing the course of differentiation of the several parts from the sheaf-like head *N*. At *m*, Fig. 2, the medullary bundle *M* has direct lateral connexion with the lower part of the region in which the gelatinous layer of volva, innermost layer of volva, and the gleba arise later. Figs. 1 and 2 are magnified 106, Figs. 3 and 4, 66.

Fig. 5. Median longitudinal section through an egg in an early intermediate stage. Differentiation of *O* has given rise to the central column *R*, the intermediate tissue *A*, and the zone *i*. The rudiment of the stipe *S* is differentiating in one side of *A*, and that of the hymenium *H* at the other side. ×36.

Fig. 6. Cluster of young basidia from the point *H*, Fig. 5. ×670.

Fig. 7. Rudiment of the stipe at its base, with adjacent tissues. From Fig. 5 at *S*. ×325.

Fig. 8. An older stage than Fig. 6 with the outer portions omitted. On account of a curvature of the stipe, the lower part of the figure—the part below *S*—had to be drawn from another section in the same series. The crowding in of basidia into the hymenial surface has thrown the latter into folds; separation of the gleba from the intermediate tissue *A* is now almost completed. ×36.

Fig. 9. Portion of the rudiment of the stipe *S* of Fig. 6 more highly magnified. ×325.

Fig. 10. Median longitudinal section through an egg in which the rudiment of the stipe is differentiating into chamber-tissue and that of partition-walls. The lower part of the figure is from another section in the same series. ×8½.

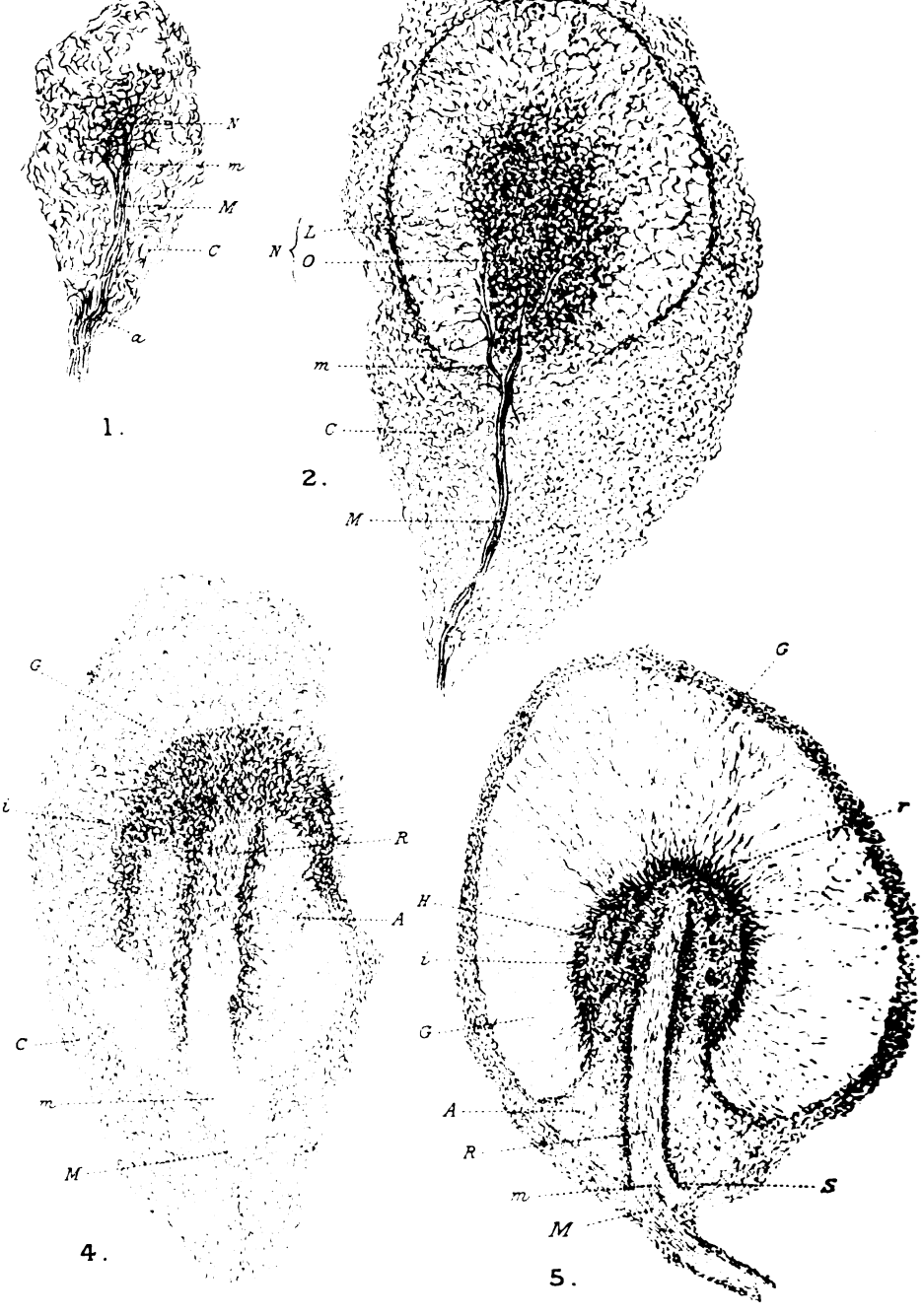
Fig. 11. Portion of rudiment of the stipe in about the stage of Fig. 10, showing the chamber-tissue *c*, *c* and the portions between and about these which become partitions of pseudoparenchyma later. ×325.

Fig. 12. Portion of the upper part of the fundament of the stipe of Fig. 10, showing a 'pit' and the tissues in its vicinity. ×325.

Fig. 13. Median longitudinal section of the stipe and adjacent parts of an egg in a late intermediate stage. ×9.

Fig. 14. Portion of Fig. 13 showing wall of stipe just below the 'spore-bearing' part, showing the connexion of the tissue *A* with the tissue *c* of chambers through passages which are perforations or openings in the external surface of the stipe after its elongation. (*Corynites Ravenelii*, B. & C., with specimens of which my material agrees, was supposed to differ chiefly from *Mutinus caninus* (Huds.) in this portion of the stipe. Comparison of this figure with Ed. Fischer's (1895) Fig. 9 of European *M. caninus* in a somewhat older stage shows no essential structural difference.)

Fig. 15. Longitudinal radial section through the wall in the 'spore-bearing' part of the stipe. Comparison with Fig. 12 shows the thickening of the wall in this part to be by the formation of a later zone of pseudoparenchyma *b* from the intermediate tissue *A*. A 'pit' *c* is on the right. ×325.



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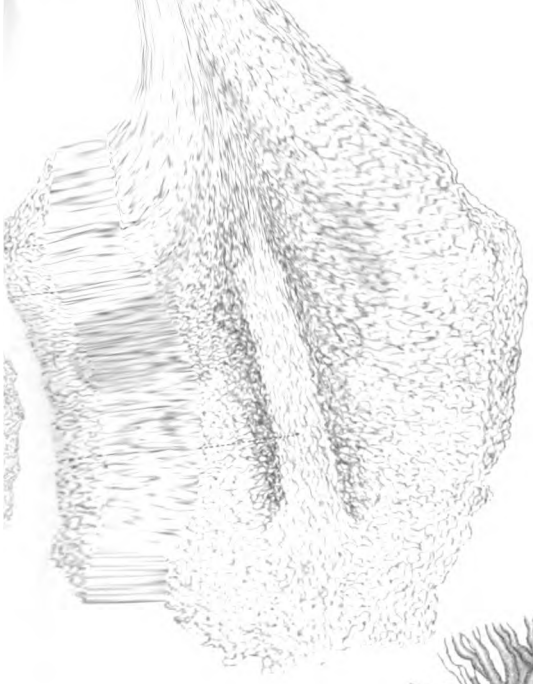

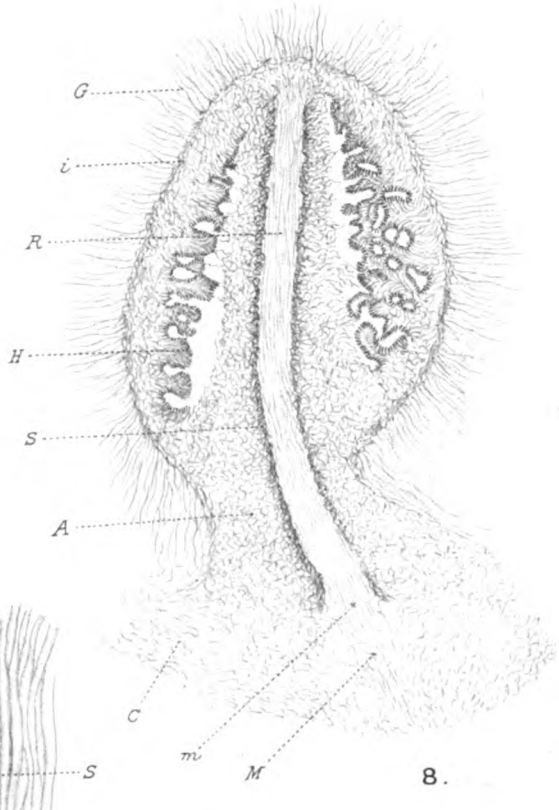

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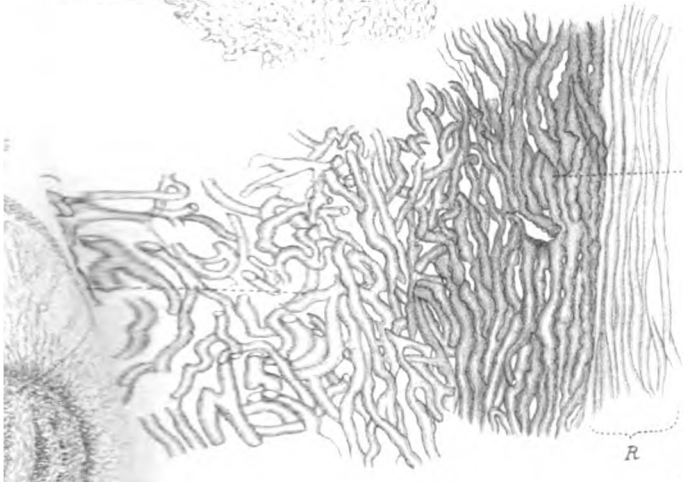

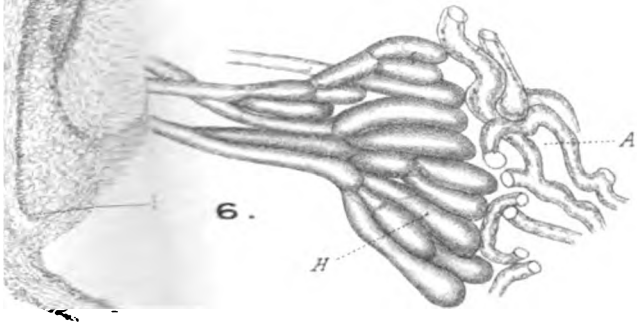

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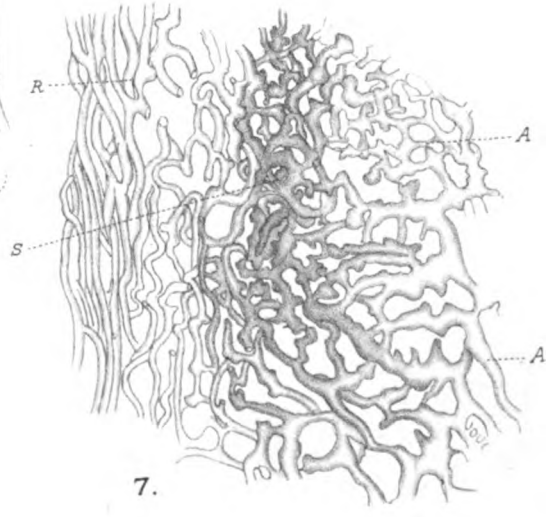

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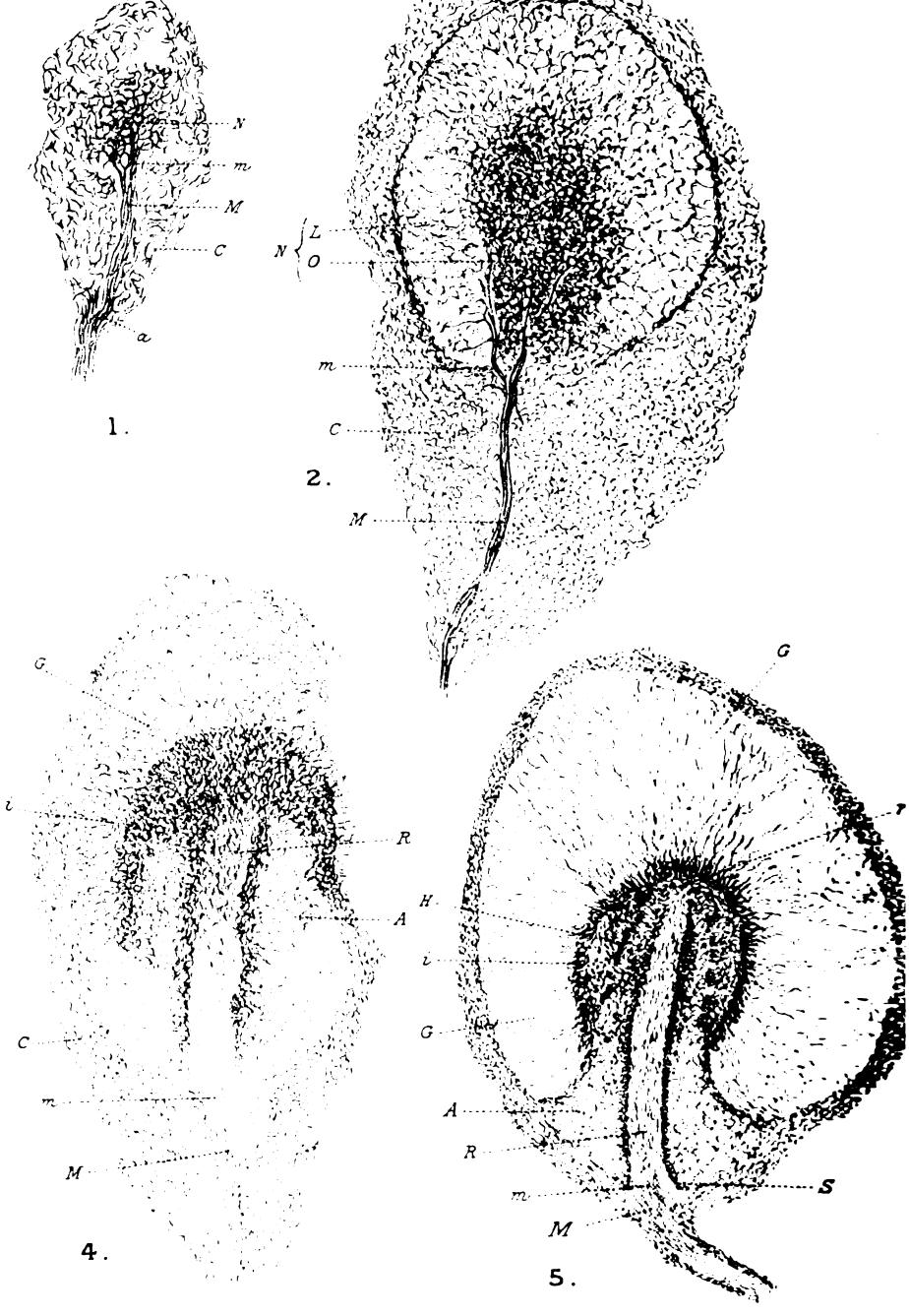

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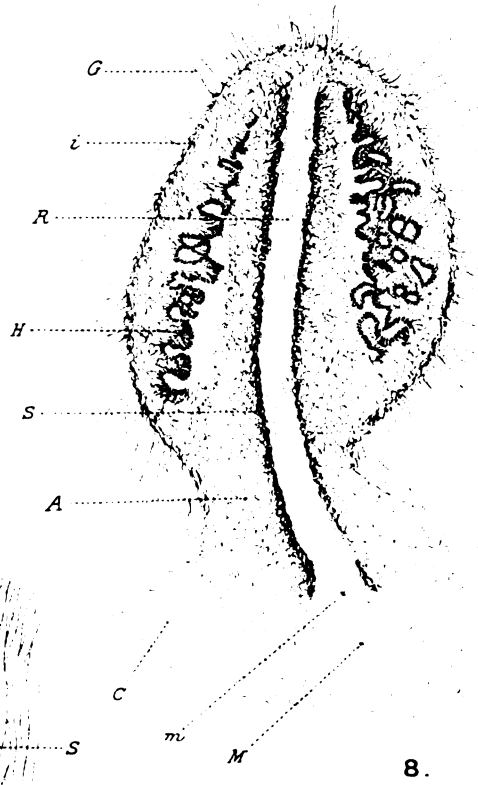

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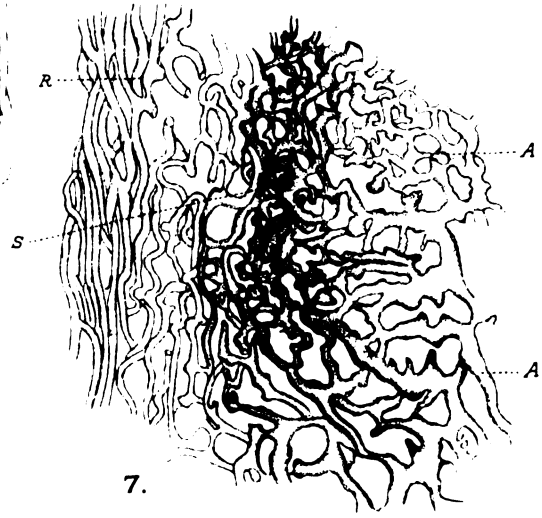

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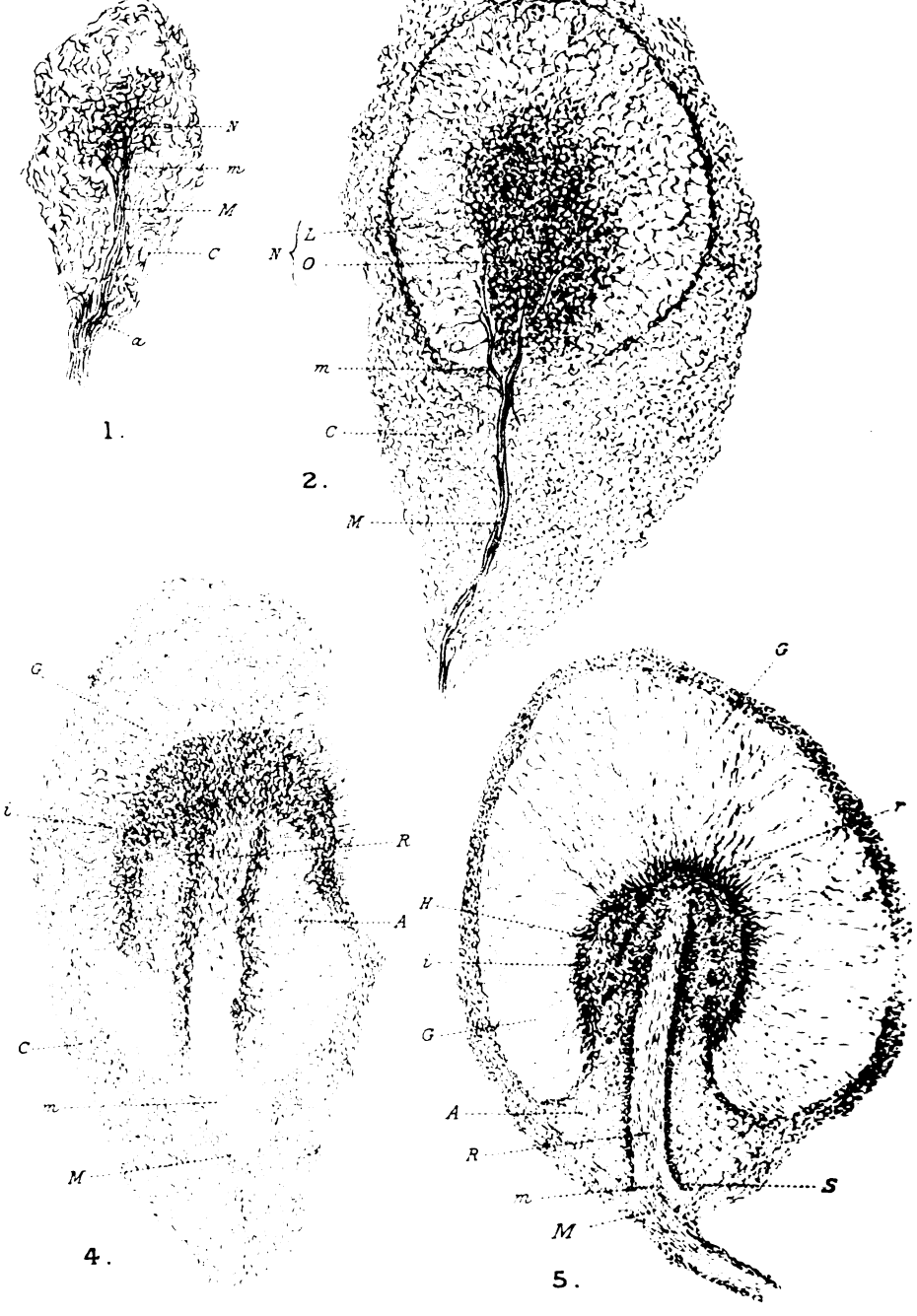

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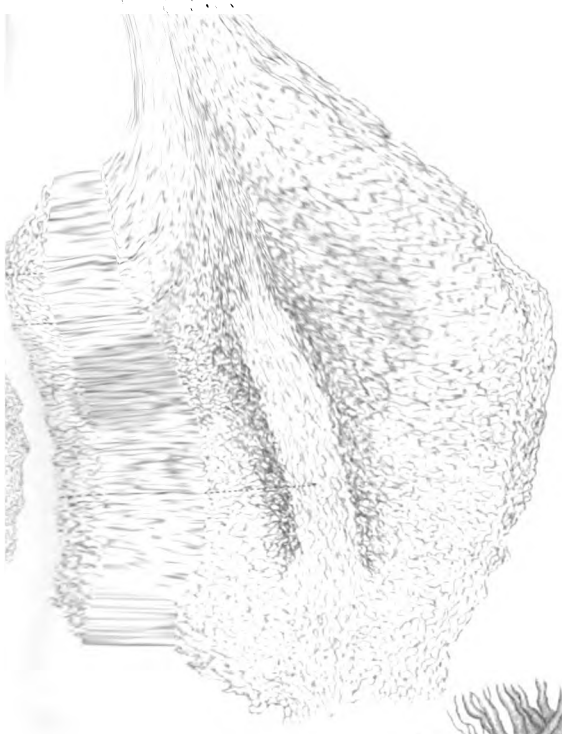

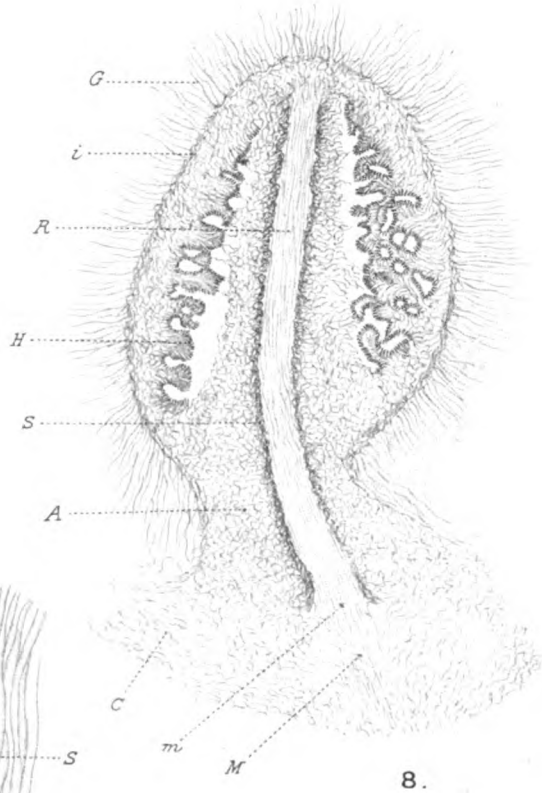

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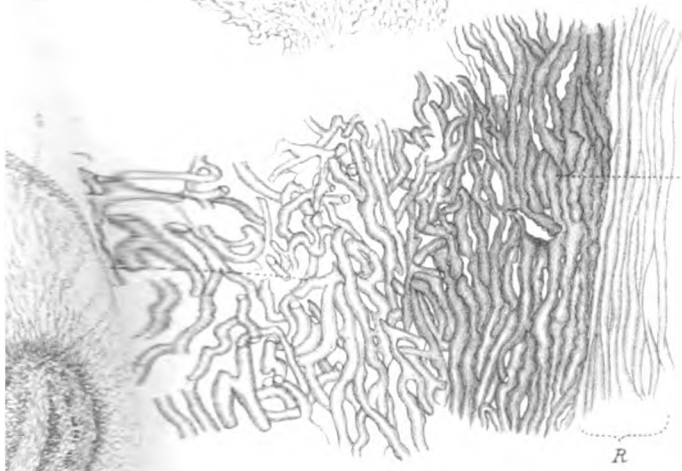

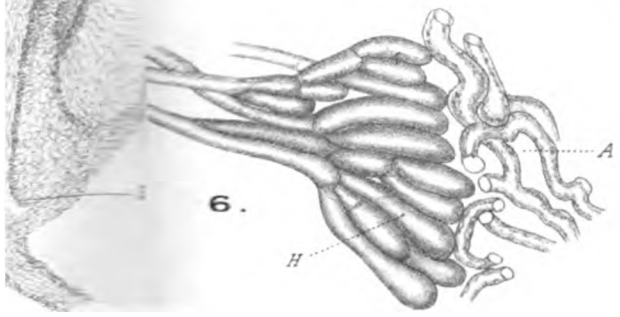

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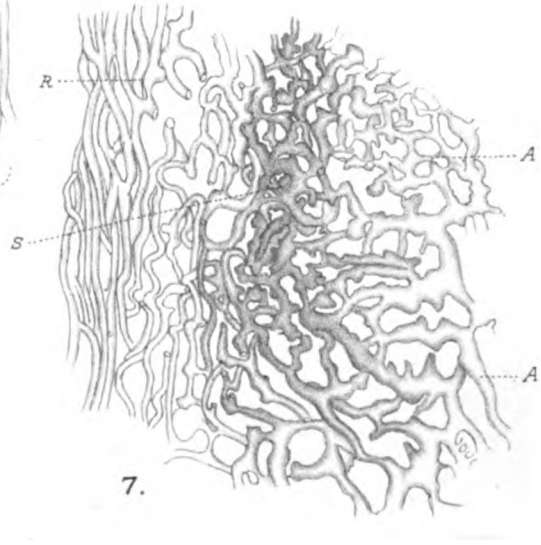

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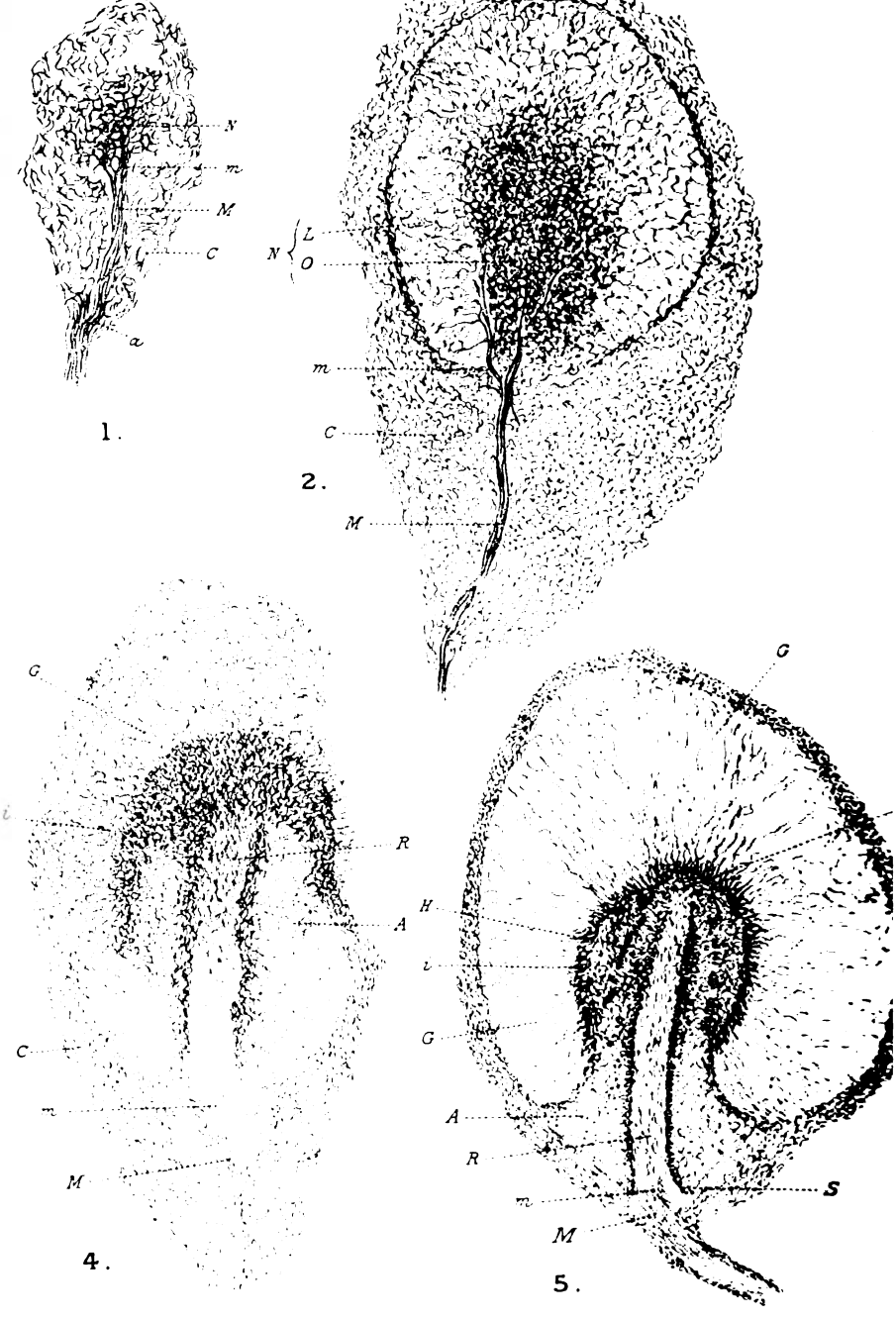

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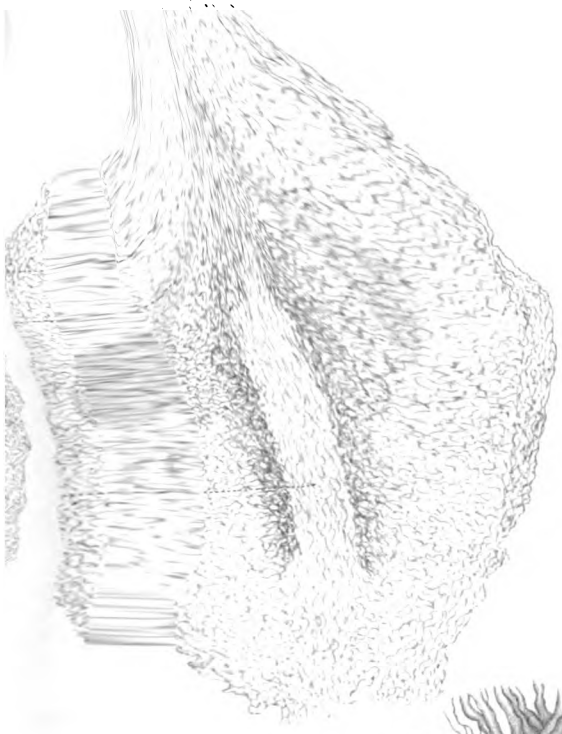

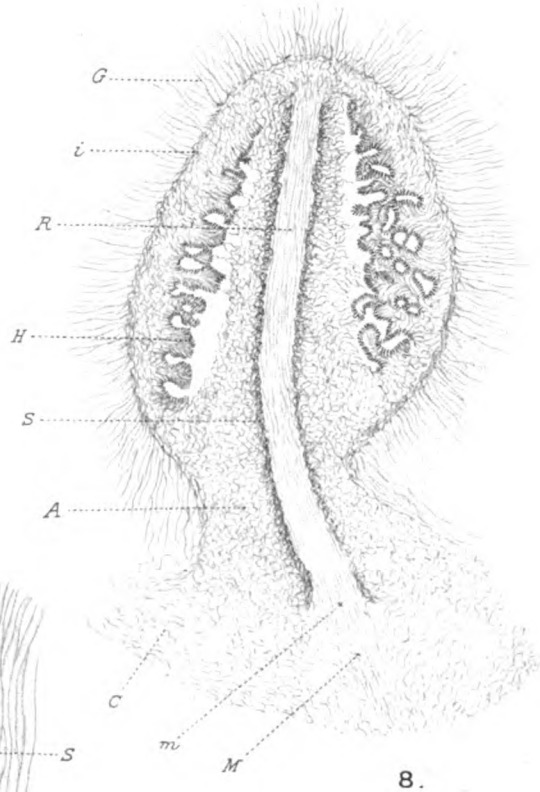

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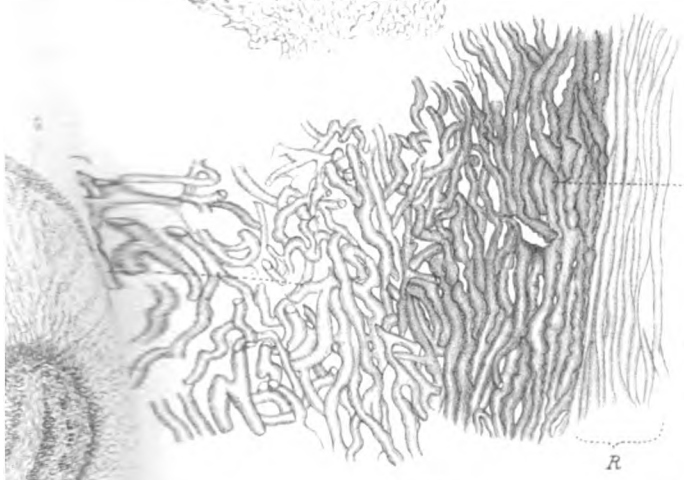

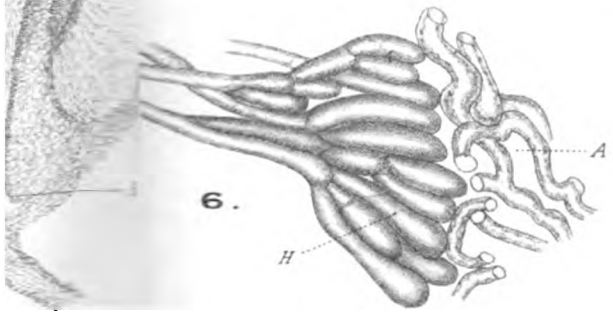

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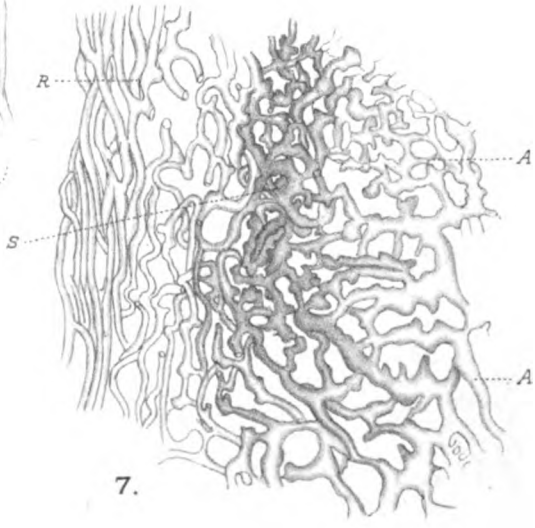

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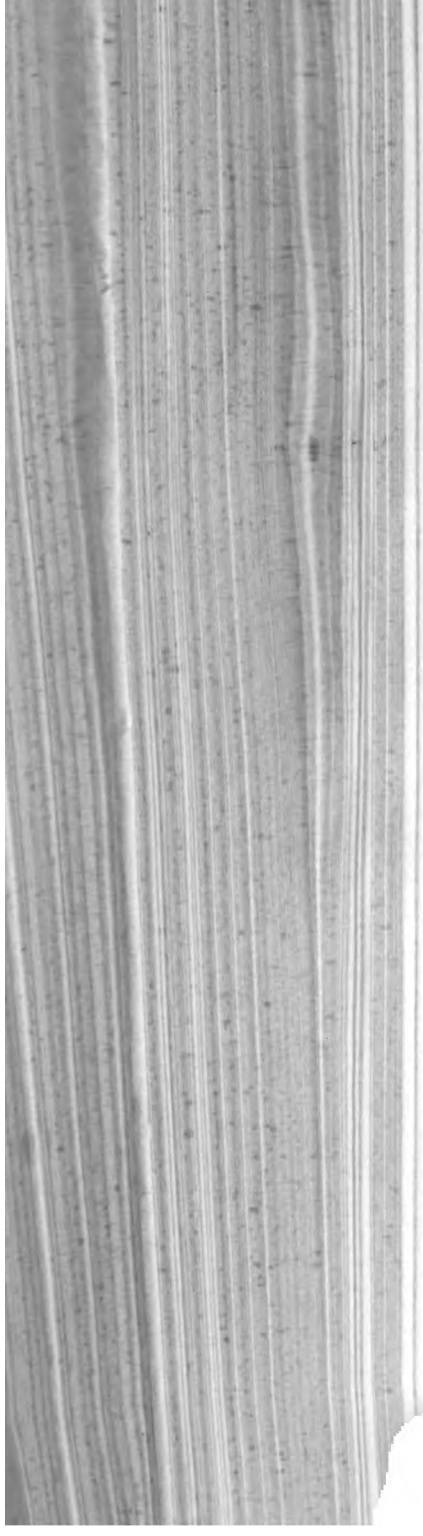

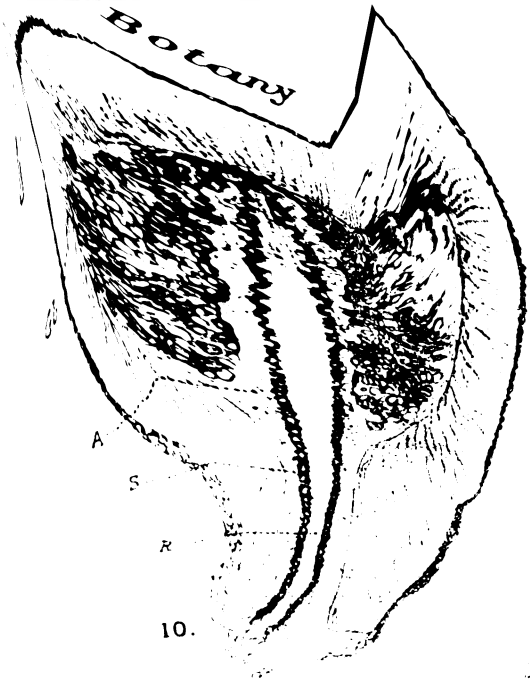

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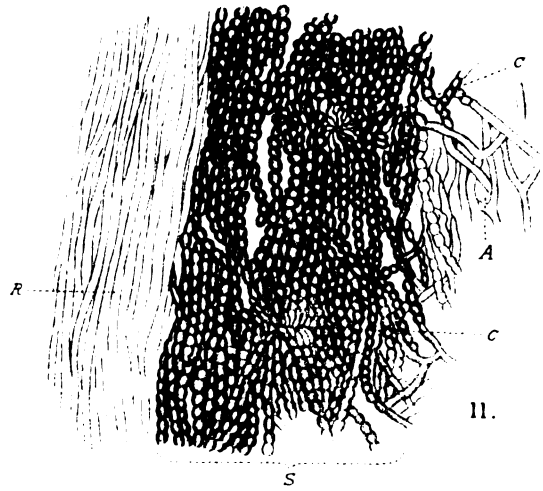

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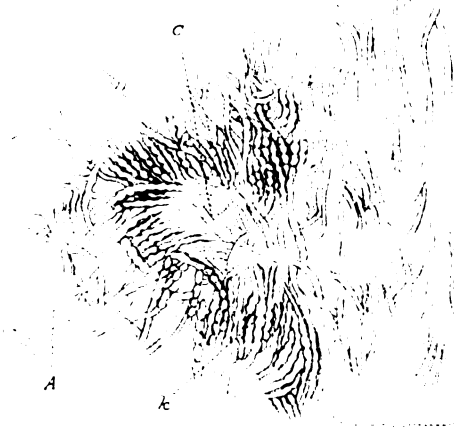

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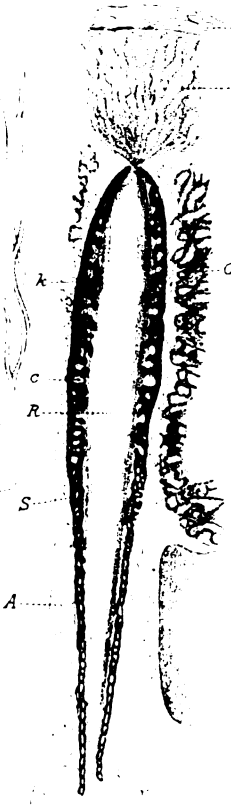

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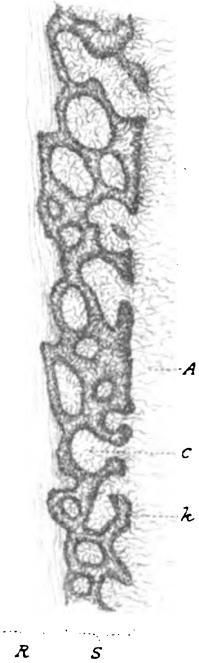

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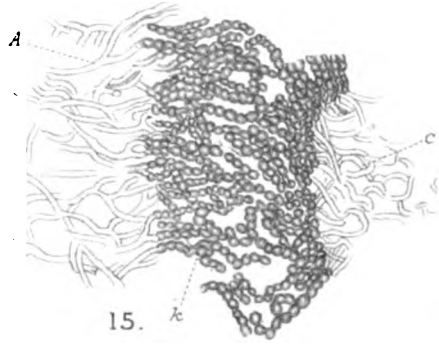

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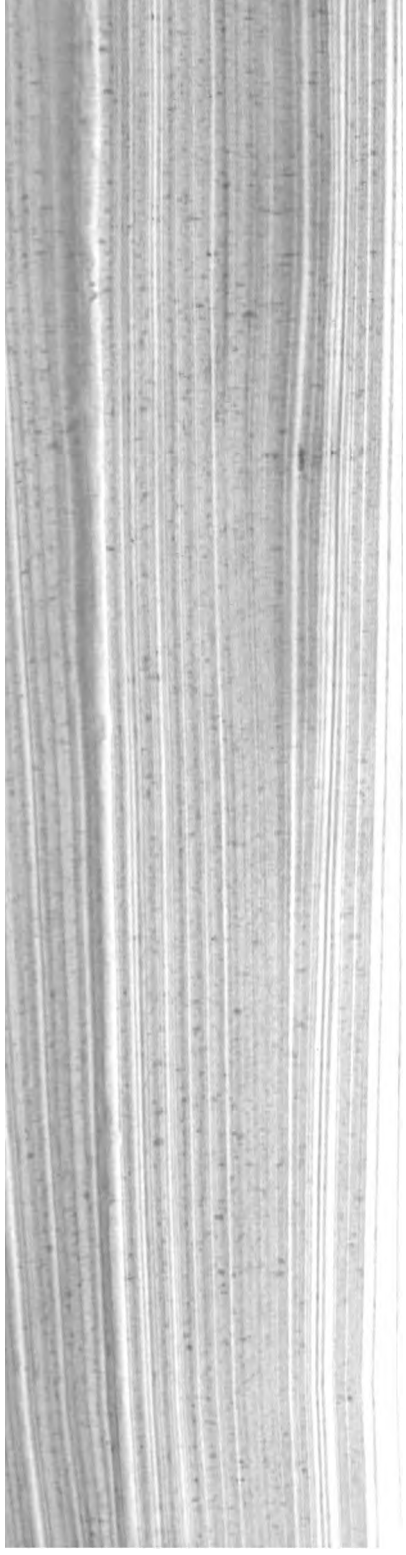

The Mechanism of Curvature of Tendrils.

BY

D. T. MACDOUGAL,

The State University of Minnesota.

With Plate XIX.

Historical and General Considerations.

In 1891 the writer began a series of experiments dealing with the features of growth and irrito-contractility of the tendrils of the Passifloreae. In 1892 a paper, concerning the principal features of the anatomy and morphology of the tendrils of *Passiflora coerulea* was published, and in the following year a second followed on the external phenomena of irritability and the coiling movements. In the course of the work many facts of significance in the determination of the mechanism of the movements, the localization of the motor zones, and the conduction of stimuli were brought to light. These facts, especially those concerning the first-named features, were much at variance with the generally accepted theories of tendril-curvature, and were not sufficiently verified by repeated observations at that time to justify their publication with my other work. With the accession of later results by myself and others, the conclusions which follow seem entirely warranted.

It may be useful in the following discussion to recall that complex organs of the higher plants which have the power of

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curvature effect the movement either by elongation of the tissues of the side of the organ becoming convex, or the contraction of a tissue on the concave side. As belonging to the first class, stems, petioles, and other organs which respond to geotropic and heliotropic stimuli, &c. may be mentioned; and to the second class, the pulvini of *Mimosa* and other plants, the tentacles of *Drosera*, the leaves of *Dionaea*, and other structures. It may as well be stated in the beginning, that my conclusions justify the assumptions of Charles Darwin, that the clasping-movements of such highly specialized, initially dorsiventral tendrils as those of *Passiflora* are produced by a contraction of the elements of a tissue on the concave (lower) side of the tendril. He says (I, p. 181): 'At first I attributed this movement to the growth of the outside; black marks were therefore made, and the interspaces measured, but I could not thus detect any increase in length. Hence it seems probable in this case and in others, that the curvature of the tendril from a touch depends on the contraction of the cells along the concave side.' A similar conclusion had previously been reached by Knight: 'The external pressure of any body on one side of a tendril will probably drive the fluid from one side of the tendril which will consequently contract, to the opposite side which will expand, and the tendril will thence be compelled to bend around a slender bar of wood or iron' (VI). From the above it is apparent that while the conclusion that tendrils effected contact curvatures by means of the contraction of the irritated side of the organ is to be accredited to Knight, yet it is clear that to Darwin must be ascribed the first comprehension of the real nature of such contraction. De Vries, by a series of comparative measurements of the growth of the convex and concave sides of tendrils (II), and later by a series of plasmolytic tests, was led to the belief that the curvature and coiling of these organs was due to an increased osmotic activity of the convex side resulting in an accelerated growth-extension of the tissues of this side. Sachs (XX) adheres in the main to De Vries' conclusions, yet he points out that a contraction of the lower side

may occur during curvature, and may amount to one-third of the original length of the organ; and furthermore admits that in consequence of the relaxation and loss of water of the parenchyma of the concave side, the elastic contraction of the cell-walls may co-operate in producing curvature or coiling.

The mechanism by which the attachment of tendrils and other climbing organs to supports is accomplished, has undergone widely different methods and degrees of development in different groups. In some instances, attachment is made by means of disc-like suckers or outgrowths adapted to the surface to which adhesion is desirable. In others, the same purpose is accomplished by means of mucilage-masses secreted by groups of specialized cells. In others, attachment is by means of curvatures which more or less encircle the support. In the last-named group the tendrils may possess the power of contact-irritability over almost their entire surface, and be able to curve in any direction; or the seat of irritability may lie on one side alone, which always forms the concavity of the curvature. After attachment to a support, the tendril generally throws its free portion into a number of loose coils, which results in shortening the distance between the shoot and support. In this manner as each internode successively develops, it is drawn up and fastened to the support. In order that this fixation may be effected economically, it is important that exact correlations should exist between the activities of the tendril and those of the shoot. It will be found, therefore, that rapidly growing plants are, in the majority of instances, furnished with tendrils of greatest sensibility and rapidity of reaction to contact, and conjointly the consequent free coiling of the tendril will be rapid and strong because of this power of rapid growth extended to the tendril. As examples of the smallness of stimuli which may cause a reaction may be mentioned the experiments of Charles Darwin (I, p. 171), in which it was found that a tendril of *Passiflora gracilis* responded when a weight of 1·23 mg. was gently laid upon it. Pfeffer found that while a stream of

water directed against a tendril would not cause a reaction, yet if some substance in the form of fine powder were suspended in the water, the repeated impact of these particles induced reaction, although their diameter was only ·04 mm., and their weight must have been only a fraction of that used by Darwin. In further experiments by Pfeffer, a rider weighing ·00025 mg. placed gently upon a tendril gave no reaction, but when it was moved by the wind, curvature resulted (XVII). It has come under the author's observation that a spider's thread, 43 cm. long, suspended above a tendril of *Echinocystis*, caused such reaction that the tendril coiled around and fastened to it. It will be found that a reaction in some of the more highly irritable tendrils of the Passifloraceae may be obtained in twenty-five seconds, while Müller (XIV) noted a curvature in the tendrils of *Cyclanthera* five seconds after stimulation. In many forms the latent period may extend over sixty to one hundred minutes.

The forces which act as stimuli upon the different types of tendrils sensitive to contact, appear to offer no means of distinction of the mechanism by which the reaction is accomplished. In general it may be said that clasping tendrils are sensitive to contact or repeated impact of solid bodies, but not to static pressure, or to shock such as that given by jarring the soil in which the plant stands, and to which *Mimosa* responds so readily, a generalization first stated by Pfeffer (XVIII, pp. 517–524). The distinct and important mechanical, anatomical, and physiological conditions to be found among tendrils which apparently react in a similar manner to identical stimuli, does not warrant the assumption that the reactions in all classes of tendrils are identical. Not only does this apply to the actual mechanism of movement, but also to that of the transmission of the effects of stimulation. Thus Pfeffer (XVIII) has found that the excision of certain tendrils, viz., *Sicyos angulatus* and *Passiflora gracilis*, was followed by the exudation of a drop of water from the exercised surfaces, a result which I have also obtained in all species of *Passiflora* and Cucurbitaceae examined. On the

other hand, this exudation does not occur in *Cobaea scandens* and other plants which exhibit a high degree of contact-irritability. When a stimulus is given to the perceptive zone, the region stimulated curves; if the stimulus is continuous the curvature strengthens, but the stimulus is not conducted farther than 2 or 3 cm. in either direction, according to Pfeffer and to my own observations. I have recently found that when tips of actively nutating tendrils of *Passiflora incarnata* were quickly cut away by means of a razor-stroke given from above, without otherwise touching the organ, a curvature of 90° was induced at a distance of 1 cm. from the cut surface, an effect which so far has not been obtained in any other plant.

The forms embraced under the general term of tendrils embrace organs of widely different anatomy and morphological derivation. This and the fact that the degree of vegetative activity, as well as the condition of, and manner in which, these organs accomplish the fixation of the plant-body to a support, will show the futility of the attempt to assign the mechanism of curvature to one type which shall apply to all those organs. The conclusions reached in this paper are therefore restricted to the actual forms of the Cucurbitaceae and Passifloreae examined. In any case, however, it is to be noted that it cannot be assumed *a priori* that the curvature of tendrils is similar to that of stems. An essential difference between the curvature of stems and of tendrils is based upon the primal fact that the curvature of a stem implies minor physiological changes in the curved organ, while this process in a tendril entails a complicated series of complete alterations in functions of the first magnitude. Furthermore, a sharp distinction is to be made between the processes of the formation of curvatures and that of loose coils. The first is a reaction to the stimuli to which a tendril will respond, while the second is consequent upon the maturity of the organ, a fact pointed out by Penhallow (XVII, p. 72). It has been repeatedly demonstrated that contact-stimuli are not conducted farther than 2 or 3 cm. at the most, and the formation of loose

coils in a tendril whose tip is attached to a support often begins at a point 25 cm. distant from the point of reception of the stimulus at the tip. The attachment of the tip of a tendril to a support by curvature exerts no direct influence on the free portion of the tendril, except by the traction exerted by the weight of the plant-body thrown upon it. The formation of the free coils of an attached and of an unattached tendril is found to differ only in so far as the effect of this mechanical strain is concerned. This is emphasized by reference to No. IV of the dynamometric tables, in which it is shown that a young tendril continued to elongate after its tip had clasped a support, and did not form coils until it had attained maturity. It is therefore necessary to exercise great care in the delimitation of the processes of curvature by which a tendril reacts to stimuli, and the processes of growth by which permanent coils are formed, in the analysis of the movements of these organs.

Results Obtained by Various Methods of Experimentation.

In the research directed toward the determination of the mechanism of curvature, two methods of experimentation have been used in securing the data upon which conclusions have been based :—First, estimation of the relative turgidity of the tissues of the convex and concave sides by means of plasmolysis :—Secondly, measurement of the distance between two points on the convex and on the concave surface of the curving organ before and after contraction. In addition to a revision of the results obtained by these methods, it is proposed to throw additional light on the subject by an examination of the structure, form, and arrangement of the cells of probable motor zones in the convex and concave sides of the tendrils before and after induced curvature.

Measurement of elongation. Independently of his plasmolytic researches, De Vries found by measurements that in encircling a support the growth in length of the convex side of a tendril

was increased, and the growth in length of the concave side was lessened. Thus in a length of 1 mm., the increase on the convex side was 1·4 mm., on the concave side ·1 mm., and in a neighbouring unstimulated portion, ·2 mm. on both sides. These figures express the total amount of change during the formation of the contact-curvatures around the support, and the ensuing growth during a period of twelve to forty-eight hours. Further, both Sachs and De Vries agree that at times an actual contraction of the concave side occurs in many instances. In the tendril of the Gourd, the actual shortening of the concave side amounted to one-third the length of the tendril at time of stimulation (I, II, XX), a fact which is not adequately explained; and it may be shown that the changes in length of the portion of a tendril which are caused by a contact with a support, and which follow upon curvature around it, have been persistently misinterpreted.

In order to obtain the general features of growth-extension, a number of developing tendrils of *Passiflora coerulea* were marked off at intervals of 1 cm. by means of india-ink and a camel's hair brush. These intervals were re-measured each day during the period of nutatory movement and irritability. The marks were placed on the convex sides of the organs, which were held firmly during measurement by means of gelatine-coated clamps in such a manner that they were not stimulated. The data obtained are given in the table below. The figures expressing the length of the portion or portions in the region of maximum growth are given in thicker type.

By a consideration of the table on p. 380 it may be seen that the entire period of development of such tendrils occupies from 110 to 130 hours. The elongation in twenty-four hours of certain portions amount to 150 per cent. of their length; the elongation of the entire tendril during the same length of time amounts to 20–40 per cent. of its length; and the elongation of the region of maximum growth must have greatly exceeded the above rate during the twelve hours inclusive of the period of most rapid growth. The comparisons of De Vries of the growth of the portion of a tendril

Table of measurements of growth of tendrils of Passiflora coerulea.

I

Length of intervals.	Base.										Tip.	Total lengths.
Mar. 2, 10 a.m.	1	1	1	1	1	1	1	1	1	1	·5	11.5 cm.
,, 3, ,,	1·6	1·6	1·9	1·9	1·9	2	2·1	1·8	1·4	1·4	·6	18·8 cm.
,, 4, ,,	1·7	1·9	2	2·4	2·3	2·5	2·9	2·3	1·9	1·9	·8	24·5 cm.

II.

Length of intervals.	Base.						Tip.						Total lengths.
Mar. 2, 10.15 a.m.	1	1	1	1	1	1	·5						6·5 cm.
,, 3, ,,	1·9	1·9	1·7	1·9	1·7	1·6	·7						11·4 cm.
,, 4, ,,	2·1	3·4	4·2	3·5	2·6	2·5	1·						19·2 cm.
,, 5, ,,	2·4	3·8	4·9	4·4	3·7	3	1·1						23 cm.

III.

Length of intervals.	Base.			Tip.								Total lengths.
Oct. 4, 9 a.m.	1	1	1	1								4 cm.
,, 5, ,,	2·2	1·6	1·2	1·2								6·2 cm.
,, 6, ,,	3·	2·5	1·7	1·4								8·6 cm.
,, 7, ,,	5·	3·8	2·	1·8								12·6 cm.
,, 8, ,,	6·6	4·7	2·5	2·3								16·1 cm.

IV.

Length of intervals.	Base.				Tip.							Total lengths.
Oct. 3, 9.15 a.m.	1	1	1	1	1	1	1					7 cm.
,, 4, ,,	2·5	1·7	2·4	2	1·9	1·3	1·2					13 cm.
,, 5, ,,	3·1	2·5	3·4	2·6	2·1	1·6	1·6					16·9 cm.
,, 6, ,,	3·2	2·6	3·7	2·7	2·2	1·8	1·8					18 cm.
,, 7, ,,	3·6	2·8	3·8	3·1	2·4	2·1	2					19·8 cm.

encircling a support and of a free portion are wholly without significance, since he has failed to indicate the positions of the zones measured with regard to the region of maximum growth, which *in the earlier stages lies in the basal portion of the tendril, and progresses forward in an oscillating manner until it may occupy a position distal to the middle of the tendril, but from which it finally recedes.*

From the numerous measurements of the elongation of the convex surface of portions of tendrils encircling a support, the writer has never found an increase greater than 50 per cent. of the maximum given above, and De Vries' theory, that curvature around a support is due to the accelerated growth of the convex side, is no longer tenable. Furthermore, if the contact-curvature of the tendril were due to growth, it might be expected that the organ would be more highly irritable in the region of greatest growth, but the *zone of greatest growth never at any time coincides with the zone of greatest irritability, which lies near the tip.*

That a difference does exist between the features of growth of a portion of a free tendril and one which has encircled a support, is quite apparent. Such differences consist in a slightly lessened elongation of the convex side and a greatly decreased elongation of the concave side, which are to be considered as consequences, not causes of contact curvature. It is to be readily seen that when a tendril is coiled around a support by any force, whether internal or external, the pressure exerted upon the concave side from the support and the internal strains would exert a very marked effect on the growth of the tissue so affected. The alterations in the growth of a curved tendril, as described by Worgitzky (XXII), are exactly such as might follow from the altered mechanical conditions. These changes consist principally in the tangential division of the epidermal cells, followed by a thickening of their walls which extends in a marked degree to the collenchyma and cortical parenchyma, accompanied by a strong development of prosenchymatous tissue. In the mean time the growth of the

convex side of the tendril has continued unchanged, except for the reaction to the slight traction exerted upon it, and the difference between the two sides in the course of a few hours becomes quite marked.

Plasmolytic Investigations.

The analysis of induced curves by means of plasmolyzing agents has been carried out at great length by De Vries upon tendrils of *Sicyos*, *Echinocystis*, *Bryonia*, *Cucurbita*, and *Passiflora*, as well as upon stems and other organs (II, III). He was led to the conclusion that the cells of the convex side of a curved organ contained a greater amount of osmotic substance than those of the opposite side, and that curvatures are produced by the excessive lengthening of the cell-membranes of this side due to the consequent increased turgidity. His first conclusion has been signally disproved by Wortmann (XXIII–XXVII), by Noll (XV), and by Kraus (VIII) so far as stems are concerned. In further consideration of the conditions of heliotropic and geotropic curvatures of stems, Wortmann believed himself to have proved that in curvature a segregation of the protoplasm on the concave side of the organ occurred, and that the membranes on this side became thickened and more resistant. The action of the uniform turgor stretched the thinner and more elastic membranes of the convex side. Kohl ascribes the curvature of stems to changes in form of the cells of the concave side due to their different elasticity in different directions (VII). Such changes of form were not described as a contraction, but rather as accompanied by an increase in volume. Noll has shown that the segregation of protoplasm on the concave side is a result that has no causal relation to curvature. His conclusions, which are strengthened by his more recent work (XV), point to the variations in the plastic and elastic extensibility of the membranes of the convex side under the regulatory power of the protoplasm as the chief factor. These conclusions, however, relate especially to curvatures of stems, petioles, &c.,

and he calls especial attention to the fact that the elongation of the longitudinal membranes is in no sense growth, since their dry weight does not increase. The only recent work dealing with the mechanism of the tendril alone is that of Penhallow (XVII, p. 75), in which he concludes that 'movements due to irritation depend upon the continued elongation of the opposite side, together with cessation of growth and contraction in the irritated parts.' In support of this conclusion, however, no additional facts, except the accentuated development of the collenchymatous tissue of the concave side, are given.

While, as noted above, it has been proved that the convex side of curving organs does not show a preponderance of osmotic substance, in opposition to the conclusions of De Vries, it is evident that such conditions would be of value only in conjunction with certain mechanical features. Moreover, I have been unable to verify the immediate facts of De Vries' plasmolytic researches on tendrils of *Sicyos angulatus*, *Cucurbita Pepo*, *Bryonia dioica*, *Echinocystis lobata*, and *Passiflora gracilis*. He found that the plasmolysis of freshly induced curvatures of stems and tendrils resulted finally in the lengthening of the radius of curvature, perhaps to such extent that the organ became straightened. At first, however, a minor amount of shortening of the radius occurred. That such may be the case with geotropically or heliotropically induced curvatures of stems and petioles under certain conditions is confirmed; but is by no means invariable, as has been shown by Wiesner (XXI). O. Müller (XIV) found that the plasmolysis of tendrils, stimulated or unstimulated, might be followed either by increased or decreased curvature, in accordance with certain unexplained inward differences.

My own results are completely at variance with those of De Vries, and differ from those of Müller in that with weak solutions plasmolysis invariably resulted finally in the shortening of the radius of curvature, in both stimulated and unstimulated organs, and this decrease continued with minor variations so long as they were alive. The following data,

taken at random from my note-book, will fully illustrate this point.

Plasmolytic experiments. Plasmolysis was effected by means of a 1 per cent. solution of potassium nitrate in a flat porcelain dish, in which the tendrils were gently placed. During the preparation they were unavoidably slightly stimulated, and their stature is noted in the first line of the description of each experiment.

I.

Dec. 24, 9.15 a.m. Tendril of *Cucurbita*, 18 cm. long, with tip curved through 300°, with radius of 1·5 cm. placed in solution.
„ 9.30 a.m. 3 complete circles with a radius of 6 cm.
„ 12 a.m. 4 circles.
„ 2.30 p.m. 3½ circles.
Dec. 25, 10 a.m. 1⅓ circle.
„ 4 p.m. Same.
Dec. 26, 9 a.m. Same.
Dec. 27, 11 a.m. Same. Placed in distilled water.
„ 2 p.m. 1⅔ circle.
Dec. 28, 2 p.m. 3½ circles.
Dec. 29, 2 p.m. Curves strengthened.

II.

Dec. 24, 9.15 a.m. A tendril of *Cucurbita*, 25 cm. long, with curve of 250° at tip, with radius of 4 cm. placed in solution.
Dec. 24, 9.30 a.m. 4 circles with a radius of ·2 to ·4 cm. were formed at the tip; entire tendril curved through 180°.
Dec. 24, 12 a.m. 5 circles at tip. Curve of tendril strengthened.
„ 2.30 a.m. 5 circles. Curve of tendril of shorter radius.
Dec. 25, 10 a.m. 6½ circles. Curve of entire tendril 320°.
„ 2 p.m. 7 circles. 1⅓ circle in entire tendril.
Dec. 26, 9 a.m. 7 circles. 1⅓ circle in entire tendril.
Dec. 27, 11 a.m. 8 circles. 1½ circle in entire tendril. Placed in distilled water.
Dec. 27, 2 p.m. Curves contracted.
Dec. 28, 2 p.m. Curves contracted.

III.

Dec. 24, 9.15 a.m. A tendril of *Cucurbita*, 20 cm. long, was placed in a solution.
„ 10 a.m. The tip curved 320° with radius of ·3 to 1·3 cm. Entire tendril curved 20°.
„ 12 a.m. No change.
„ 2.30 p.m. Curves decreased to 240°. Entire organ straightened.
Dec. 25, 10 a.m. 1⅓ circle.
„ 4 p.m. Same.
Dec. 26, 9 a.m. Same.
Dec. 27, 11 a.m. 2⅔ circles. Placed in distilled water.
„ 2 p.m. Curve 3½ circles.
Dec. 28, 2 p.m. Curve contracted.

IV.

Dec. 24, 9.15 a.m. Tendril of *Cucurbita*, 20 cm. long, placed in solution.
„ 10 a.m. Tip of main tendril in 3 circles of radius of .4 to 1 cm. 1½ circle in branch.
„ 12 a.m. No change.
„ 2.30 p.m. No change in main tendril. Curve of 300° in branch.
Dec. 25, 10 a.m. 1½ circle in main tendril. Curve of 300° in branch.
„ 4 p.m. Curve of 1⅔ circle in main tendril. Curve of 300° in branch.
Dec. 26, 9 a.m. Same in main tendril. 1 circle in branch.
Dec. 27, 11 a.m. Curve of 350° in main tendril. 2 circles in branch. Placed in distilled water.
Dec. 28, 2 p.m. 3 circles in main tendril. 2½ circles in branch.
Dec. 29, 2 p.m. No change.

From the long continuance and variations in changes in the stature of the tendril, both in the plasmolysing fluid and in distilled water, it is evidently unsafe to draw conclusions as to the initial osmotic conditions, since the resulting movements

must also be largely influenced by the degree of development of the mechanical tissues, and the elastic extensibility of the walls of the mobile tissues. In order to ascertain the conditions resulting in the active cells, tendrils of *Passiflora* which had been plasmolysed in 2, 3 and 4 per cent. solutions of potassium nitrate, and allowed to remain in the solution until killed, were carefully hardened by the use of series of alcohol-mixtures and stained. In the parenchymatous tissues of both the concave and convex sides of the tendril, the cells were strongly plasmolysed, so that the protoplasts were in a large number of instances torn completely from the wall. The differences in form and size of the cells of the convex and concave sides make comparisons difficult, but it was apparent that the outer rows of parenchymatous cells of the concave side immediately internal to the collenchyma had lost their oblong ovoid form and become irregularly globoid. In these cells the ectoplasmic layer was barely separated from the wall, while in the cells of the convex side, whose walls retained their normal stature, the separation was quite distinct, and the protoplast enclosed no more than a half or two-thirds of the volume of the cell. The only satisfactory explanation of such conditions is that the solution, while it simply plasmolysed the cells of the convex side which retained their normal stature, acted as a stimulus on the contracted cells of the concave side, upon which the ectoplasm became extremely permeable to water and allowed the sap to escape. The elastically extended walls of the plasmolysed and mechanical tissues contracted simultaneously, indirectly causing the curvature of the tendril, immediately after immersion in the plasmolysing fluid. The solution afterwards penetrated the wall of the contracted cells sufficiently to slightly plasmolyse them. The continued curvature of the organs, when placed in distilled water, can only be due to conditions similar to those prevailing in loosely coiling mature tendrils, the most important feature of which is the loss of the power of growth and turgidity of the parenchymatous cells of the concave side.

Strains exerted in Curvature and Free Coiling.

Still farther insight into the amount and character of the forces concerned in the rapid formation of a curvature in response to contact, and the formation of free coils, is offered by the data obtained from a careful measurement of the strains set up during such processes. To this end tendrils of *Cucurbita* and *Passiflora* were placed in horizontal and upright positions with the base firmly fixed to a support, and extended as far as possible, and the tip attached to a Vöchting's dynamometer (X) in such manner that the strain set up by any curvature in the organ would be directly indicated on the sliding scale carried by one of the compass arms of the instrument. In such experiments attention was also given to the relative effect of the turgidity of the plant upon the strain exerted by the free coils, the time necessary for the formation of the coils, and the effect of irritation upon the coiling portions. A large number of such tests were made, of which those detailed below are fairly illustrative.

I.

Sept. 25. After fastening the stem of a Gourd firmly to an iron post at the base of a tendril 35 cm. long, the tip was placed loosely in the attachment hook of the dynamometer, so that the body of the tendril was loosely held between the stem and the dynamometer. After it had formed a strong curve at the point of attachment with the dynamometer, the latter was fastened firmly in a horizontal position, with the tendril vertically extended, and exerting a strain of less than 1 gram.

Sept. 27, 8 a.m. A series of wide coils were forming, inducing a strain of 2·5 grams; at 6 p.m. 8·5 grams. The radius of the coils had increased from ·5 cm. to 2 cm.

Sept. 27, 8 a.m. The strain amounted to 12·5 grams. The sun shone directly upon the tendril (and upon the terminal portion of the plant, 2 M. in length, all day) during the forenoon.

Sept. 27, 10 a.m. The strain had decreased to 11 grams, and with the shading of the tendril it immediately began to increase.

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Sept. 27, 8 a.m. The strain amounted to 12·5 grams. The sun shone directly upon the tendril (and upon the terminal portion of the plant, 2 M. in length, all day) during the forenoon.

Sept. 27, 10 a.m. The strain had decreased to 11 grams, and with the shading of the tendril it immediately began to increase.

Sept. 27, 12.30 noon. The strain amounted to 12·5 grams. The temperature of the plant-house at this time rose to 27° C., the leaves of the whole plant assumed a wilted drooping position, and the strain at 3 p.m. fell to 11·5 grams. Four litres of water were then applied to the soil about the roots. After pouring on the water an increase of tension began, which at 3.15 amounted to 12 grams, and at 3.30 to 13 grams; 6 p.m. to 14 grams; 9 p.m. to 15 grams, which was the capacity of the dynamometer. The strain remained fixed at that point at 8 a.m. on the 28th.

II.

Sept. 14. A tendril of *Passiflora incarnata* was attached by the hooked tip to the dynamometer, which denoted a strain of ·5 gram. After twenty minutes, when the strain was zero, the entire under surface of the tendril was stimulated by rubbing. A tension of ·2 or ·25 gram was set up, and the organ was thrown into a number of curves which gave it an undulating outline. In an hour this tension had disappeared.

Sept. 15, 8 a.m. The dynamometer showed a strain of ·25 gram. The hooked tip had contracted still further, elongated and encircled the hook of the dynamometer.

At 5·30 p.m. the strain had increased to ·5 gram, and the tendril had formed a number of loose coils.

III.

Oct. 9, 1 p.m. A tendril of the Gourd was fastened in an inverted position with the tip attached to the dynamometer, with a strain of ·75 gram and a temperature of 30° C.

Oct. 10, 4 p.m.	Sunny	Temperature	22° C.	Readjusted to	·5	grm.
Oct. 11, 8.30 a.m.	„	„	16° C.	Tension	3	„
„ 12 a.m.	Cloudy	„	25° C.	„	4·5	„
„ 3 p.m.	„	„	22° C.	„	7	„
Oct. 12, 8 a.m.	Sunny	„	19° C.	„	9·5	„
„ 10 a.m.	„	„	20° C.	„	9·5	„
„ 4 p.m.	„	„	28° C.	„	10	„
Oct. 14, 10 a.m.	„	„	32° C.	„	7	„
Oct. 15, 8 a.m.	„	„	14° C.	„	8	„

IV.

Oct. 6, 8 a.m. A tip of a tendril of *Cucurbita* fastened to dynamometer.

Oct. 6, 4 p.m.	Sunny	Temperature	14° C.	Tension	1·5 grm.
Oct. 7, 10 a.m.	„	„	14° C.	„	3 „
„ 11 a.m.	Cloudy	„	14° C.	„	3·5 „
„ 12.30 a.m.	„	„	16° C.	„	3·5 „
„ 4.30 p.m.	„	„	12° C.	„	4 „
Oct. 8, 8 a.m.	Sunny	„	13° C.	„	4 „
„ 10 a.m.	„	„	23° C.	„	3 „
„ 11 a.m.	„	„	24° C.	„	3 „

A rapid increase in length made a readjustment of the dynamometer necessary at 4 p.m. The tension was made the same as in the last observation.

Oct. 9, 8.30 a.m.	Sunny	Temperature	15° C.	Tension	2 grm.
„ 10 a.m.	„	„	24° C.	„	5·5 „
„ 12 a.m.	„	„	35° C.	„	6 „
„ 3 p.m.	„	„	35° C.	„	7·5 „
„ 6 p.m.	Sunset	„	21° C.	„	11 „
„ 10 p.m.	Dark	„	35° C.	„	15 „

V.

Oct. 20, 10 a.m. Branch of tendril of *Cucurbita* attached in horizontal position to dynamometer.

Oct. 20, 4.30 p.m.		Temperature	29° C.	Tension	2 grm.
Oct. 21, 9 a.m.		„	20° C.	„	3 „
Oct. 22, 8 a.m.	Sunny	„	29° C.	„	4·5 „
„ 11 a.m.	Leaves wilted	„	36° C.	„	3·5 „
„ 12 a.m.	Cloudy	„	31° C.	„	4·5 „
„ 1.30 p.m.	„	„	27° C.	„	5 „
„ 4.30 p.m.	„	„	20° C.	„	6 „
Oct. 23, 8 a.m.	„	„	31° C.	„	4·5 „
„ 3 p.m.	Sunny	„	36° C.	„	5 „
„ 5 p.m.	„	„	23° C.	„	6 „
Oct. 24, 8 a.m.	„	„	17° C.	„	5·5 „
„ 11 p.m.	„	„	28° C.	„	5 „

VI.

Oct. 31, 8 a.m. Tendril of *Cucurbita* in horizontal position attached to dynamometer. (Cloudy during the entire exp.)

Oct. 31, 10 a.m.	Temperature	18° C.	Tension	1	grm.
Nov. 1, 8 p.m.	„	20° C.	„	3·5	„
„ 5 p.m.	„	21° C.	„	4·5	„
Nov. 2, 8 a.m.	„	18° C.	„	7	„
„ 11 a.m.	„	17° C.	„	7	„
Nov. 3, 10 a.m.	„	12° C.	„	6·5	„
Nov. 4, 3 p.m.	„	17° C.	„	6	„
Nov. 6, 1 p.m.	„	18° C.	„	6	„

VII.

Oct. 24, 8 a.m. A tendril of *Cucurbita*, 28 cm. long, attached in horizontal position to dynamometer.

Oct. 24, 11 a.m.	Sunny	Temperature	28° C.	Tension	1	grm.
„ 2 p.m.	„	„	30° C.	„	1·5	„
„ 3 p.m.	„	„	25° C.	„	2	„
Oct. 25, 8 a.m.	„	„	19° C.	„	4·5	„
„ 11 a.m.	„	„	28° C.	„	5	„
„ 5 p.m.	Dark	„	19° C.	„	9	„
Oct. 26, 8 a.m.	Sunny	„	17° C.	„	14·5	„
„ 11 a.m.	„	„	36° C.	„	10·5	„
„ 5 p.m.	Cloudy	„	23° C.	„	13·5	„

VIII.

Oct. 16, 8 a.m. Tendril of *Cucurbita* attached in perpendicular position to hook of dynamometer.

Oct. 16, 11 a.m.	Temperature	38° C.	Tension	2	grms.
„ 4 p.m.	„	30° C.	„	3	„
„ 6 p.m.	„	26° C.	„	4	„
Oct. 17, 10 a.m.	„	23° C.	„	5	„
„ 12 a.m.	„	23° C.	„	5	„
„ 6 p.m.	„	21° C.	„	7	„

Oct. 18, 8 a.m.	Temperature 13° C.	Tension 7 grms.
,, 11 a.m.	,, 25° C.	,, 6 ,,
,, 5 p.m.	,, 24° C.	,, 7 ,,
,, 6 p.m.	,, 20° C.	,, 8 ,,
Oct. 19, 8 a.m.	,, 18° C.	,, 10 ,,
,, 10 a.m.	,, 24° C.	,, 8 ,,
,, 1 p.m.	,, 34° C.	,, 9 ,,
,, 4 p.m.	,, 33° C.	,, 9 ,,

From the data given in the preceding tables it may be seen that the fixation of any portion of a tendril to a support does not induce the formation of coils in the free portion of the organ by transmission of a contact-stimulus. This is demonstrated by the fact that in a large number of experiments, after the tip of the tendril had clasped the dynamometer-hook, its extension in length made an alteration in the distance from the base of the tendril necessary (see Exp. IV). The formation of coils in the free portion of the tendril depends upon inequality of growth of the convex and concave sides, and may be induced by an artificial mechanical traction, or by the weight of the stem depending upon it, only when the period of growth of the organ has been nearly completed. The application of brief contact-stimuli to the irritable surface of the free portion of the tendril, in no wise modified the manner and time of the formation of the free coils. Such stimuli caused the formation of weak curves exerting an additional strain of ·2 to ·4 grams, which disappeared with the removal of the stimulus. The increase in the strain exerted upon stimulation speaks simply of a release of tension of the concave side and of a similar amount of unbalanced tension of the convex side. On the other hand, the decrease in the strain exerted by a tendril of a wilted plant must be due to the lessened tension of the entire organ due to an insufficient water supply. The decrease of strain exerted by a fully matured organ may be ascribed to the loss of turgidity of the parenchymatous cells. The lesser strain of old tendrils is due entirely to the elasticity of the mechanical elements which were built up while the organ was held in a coiled position by the turgidity of the

active cells. After a tendril has reached the end of the grand period of growth, the formation of free coils in either an attached or unattached tendril of *Cucurbita* occupies from ten to eighty hours, and is modified only by the traction exerted by the weight of the stem which it supports. The manner in which strains may arise or be induced, and their continuance, point to the conclusion that curvature in response to contact-irritation and the formation of free coils are entirely distinct physiological processes

Anatomy and Morphology.

The most important facts in the localization and determination of the action of the motor zones would be those obtained by a consideration of the anatomy and morphology of the tendril with especial reference to the structure and arrangement of the parenchymatous tissue lying between the vascular and epidermal systems. Curiously enough an examination of the changes in structure and stature of these cells for the foregoing purpose has not heretofore been made.

Although the work in hand has been extended to cover a large number of species of Cucurbitaceae and Passifloreae, it will be most convenient to restrict the discussion to the above-named features of *Passiflora coerulea*, *P. incarnata*, and *P. Pfordti*, which the author has had under more or less constant observation for several years. The tendrils of these species are tapering filamentous organs of ovoid cross-section, attaining a length of 20 to 30 cm., and a diameter at base of 2 mm. decreasing at the tip to 1 mm., which for a length of ·5 cm. is curved into a characteristic hook-form. The tendril makes its appearance as a lateral conical projection from the growing point, in the axil of a leaf (XXII), and shortly by reason of the accelerated growth of the periblem, there is formed, on the summit of the cone, a cup-shaped depression, which becomes more and more marked, and by reason of the excessive growth of the convex (upper) side of the tendril it faces laterally (or downward) when the tendril

has reached a length of 3 cm. This unequal growth also accounts for the hooked-shape formation of the tip. The cup-shaped cavity seems to be confined to tendrils of Passifloreae, and so far as the observation of the author extended, it has been found to subserve no mechanical or other use, although it is possible to imagine it might prevent to a slight extent the slipping of a tendril away from a support before a curvature had been formed. It is altogether probable on the other hand that this cup-shaped cavity is entirely a vestigial structure in the transformation of a leafy branch into a tendril; a conclusion justified by the fact that I have, in several instances, found the walls of this cup extended into a leaf-like lamina. Similar structures were observed by von Mohl (XIII). This is in accordance with the conclusions of Russell (XIX) as to the morphology of the tendrils of the Passifloreae. 'En résumé, la vrille des Passiflores représente un rameau axillaire modifié qui peut avoir à sa base plusieurs rameaux secondaires dépourvus de feuilles axillantes. Celui qui représente la première ramification se développe toujours, et peut donner, soit un rameau feuillé remplacant le rameau axillaire transformé en vrille, soit un pédicelle floral, simple ou ramifié. Les autres qui n'existent que dans la région florifère deviennent des pédicelles floraux ou bien avortent en tout ou en partie.'

While no attempt has been made to determine the changes in the tissue directly connected with the curvature alone of tendrils, yet much attention has been given to their general anatomy and to the alterations of structure consequent upon curvature and coiling. Penhallow has described the anatomy of the tendrils of *Cucurbita maxima* and *C. Pepo*, and of *Vitis cordifolia* (XVII); A. Fischer, the general structure of the tendrils of Cucurbitaceae (IV); and Otto Müller has devoted considerable attention to several species of the Cucurbitaceae (XIV). Worgitsky has recently made a comprehensive investigation into the comparative value of the mechanical tissues and their arrangement in a large number of species representing many genera (XXII) inclusive of *Passiflora*,

A clear comprehension of the results presented below necessitates the delineation of the general features of structure of the tendril of *Passiflora coerulea*.

Structure of the Tendrils of Passiflora (IX).

An examination of these organs was first made by Hugo von Mohl (XIII, p. 3, Figs. 4 and 5, Pl. II); he describes the structure, as seen in cross-section, as follows: 'Die Rinde besteht aus regelmässigen grünen Zellen (a); an der Stelle wo sie mit dem jungen Holze (b) zusammenstösst, liegen die Baströhren in getrennten Bündeln (c). Der Holzkörper besteht aus feinen Spiralgefässen und dickwandigen Holzzellen, die Markzellen (e) sind in die Länge gestreckt;' and later by Worgitsky (XXII), in which work it is to be noted that chief attention was paid to the distribution of the tissues in cross-section.

In longitudinal section the epidermal cells are somewhat uniformly rectangular in outline, with the long axis parallel to the long axis of the tendril. The protoplasts occupy a large proportion of the cell cavity. The nucleus, especially on the concave side, generally lies against the inner wall. The protoplasm is richly granular, but more markedly so in the concave side; moreover the proportion of granular substance gradually increases in the cells of the concave side from the base to a point near the tip, in a manner corresponding quite exactly with the degree of irritability exhibited by the respective areas included. This fact may be demonstrated by staining longitudinal sections in eosin and haematoxylin as I have previously shown (IX). When so prepared, the gradation in density is appreciable with a magnification of 50 diameters. The outer walls of the concave side are slightly arched outwardly, and are only cuticularized after maturity. These convexities must greatly increase the delicacy of perception of stimuli. Any solid body applied to their surface would in consequence touch the crest of these convexities, and the shock of impact, or pressure of contact,

would be communicated directly to the ectoplasmic layer on the opposite (inner) side of the wall of the cells touched, which under ordinary circumstances would be several in number. The failure of the impact of liquids at ordinary temperatures to produce a reaction, is probably due both to the smallness of shock and the fact that it is received over the entire outer surface of the wall. Pfeffer has noted an extension of the ectoplasmic layer into the outer wall of the epidermal cells of the irritable surface of tendrils of *Cucumis sativa* (XVIII, p. 525), a structure absent from any other material examined by him or myself. The epidermal layer is broken in places by stomata, which communicate with the cortical parenchyma by very narrow intercellular passages. The collenchyma-layer is one, two, or three cells in thickness. The tangential walls are much heavier, and show a number of perforations. No great difference in these respects can be made out between the concave and convex sides. The protoplasm of the concave side almost completely fills the cells, and is very densely granular, in marked distinction to that of the convex side. Moreover the contents of the collenchyma-cells of the concave side, when treated with a mixture of eosin-haematoxylin, take on a yellowish violet tinge, due to the absorption of the eosin by the innumerable minute granules which it contains. The marked difference between the contents of the collenchymatous tissue of the two sides, favours the presumption that the densely granular condition of those of the concave side bears some connexion with the transmission of impulses transversely or longitudinally; although it is not to be forgotten that the granular contents are used during the thickening of the cell-walls, after continued curvature, and their presence might be solely as reserve substance. The parenchymatous tissue internal to the collenchyma exhibits marked differences in form and behaviour. The parenchyma cells of the concave side are longer in proportion to their width than those of the concave side, and their absolute length is no greater than those of the convex side (IX, Pl. XIV, Figs. 2, 3), while their diameter is less.

Furthermore the cells of the concave side are provided with tapering ends, and the end walls are united over a small area only. As a consequence of this arrangement, the intercellular spaces are large. The cells of the convex side are united more completely across the ends, and the intercellular spaces are relatively smaller. The protoplasm of the elements of this tissue in the concave side is much more densely granular, and occupies a much greater proportion of the cell-cavity than in the convex side. The parenchymatous cells are arranged in four or five layers on both the concave and convex side of the tendril, but owing to the greater diameter of the cells of the convex side, the tissue of this side attains a marked preponderance in thickness. The two outer layers are furnished with chloroplasts varying from 6 to 10 in number in each cell. The parenchymatous tissues of both the cortex and pith exhibit marked infoldings of the walls. In tendrils exposed to strong sunlight, a reddish cell sap is found in numerous cells both in the epidermis and cortex of the convex and concave sides. Previous to the maturity and coiling of the tendril, the prosenchymatous and tracheary tissue are not developed to such extent as to be of great mechanical value. The arrangement of the vascular elements conforms to the outline of the tendril and its mechanical needs. The chief mechanical element during the irritable stage of the tendril is the collenchyma-cylinder, which as well as the epidermis and vascular tissue must be in a state of extreme elastic extension, due to a small extent to their own turgidity and to the excessive turgidity of the parenchymatous cells. Under such circumstances curvature might result from an increase in the extensibility of the walls of the convex side, or by a loss of turgidity or by an active contraction due to the loss of elasticity of the walls of the cells of the concave side. The contour of the parenchymatous cells of the two sides after plasymolysis as already described before curvature, after curvature, and in a mature coil which has fastened around a support, offers some conclusive evidence in favour of the latter view.

Action of the parenchymatous cells in plasmolysis, curvature, and coiling. During the course of my work on the morphology and anatomy of the tendrils of *Passiflora coerulea* I had occasion to examine many hundreds of cross and longitudinal sections of the irritable portions which had been killed by immersion in acetic alcohol. The changes in the stature of the cells of the concave side were marked and significant, and seem to offer a complete solution of the problem. When active tendrils are placed in the acetic alcohol solution, they execute a number of rapid oscillating curvatures and are shortly fixed by the action of the reagent. In such tendrils the parenchymatous cells had all been slightly plasmolysed before fixation. The outer two or three layers of the cells of the concave side had been moreover diminished in length from 20 to 40 per cent in size, and taken on a globoid form quite similar to that attained in simple plasmolysis. By reason of such change of volume and shape, the surfaces of contact with the neighbouring cells had been greatly diminished. The contraction of the protoplast around the central vacuole had almost obliterated it. At the same time numerous aggregation-bodies had been formed. On the convex side of the organ, the more attenuated protoplasts were not so much affected, and only modifications of minor importance had occurred, and the aggregation-bodies were noticeably absent. It seems entirely warrantable to connect the structure and behaviour of the cells of the concave side with the curvature of the tendril, especially since these features correspond almost exactly to certain characteristics of other known contractile cells. The action of the stimulus upon the cells of the motor zone, which are highly turgid, doubtless results in an increase of the permeability of the protoplasm and the consequent escape of water into the intercellular spaces. The outward passage of the fluid is facilitated by the highly-porous condition of the walls. This loss of water allows the elastic contraction of the highly-stretched walls of the cells affected, and also of an amount of contraction of the collenchymatous epidermal and vascular

tissues equal to the stretching force previously acting upon them. The contraction is not due directly to a muscular contraction of the protoplasts as has been suggested by MacFarlane in connexion with his work upon *Dionaea* (XI), but to the release of an expansive force exerted up to the moment of the movement of contraction. The action of the stimulus in either instance doubtless results in the sudden release of chemical energy, due to new affinities set up, and is accompanied by the excretion of carbon dioxide, water, and other substances. The great volume of the intercellular spaces of the motor tissues would admit of an extension of a much greater quantity of water than that necessary to allow the ordinary amount of contraction, and the return of the motor cells to their normal stature would be accompanied by the reabsorption of the intercellular fluid. *The greater density of the protoplasm of the concave side, the richness of its granular contents, the formation of aggregation-bodies, and the alterations in stature and volume of the parenchymatous cells of the concave side, lead to the conclusion that it is to the action of these cells that contact curvatures are due.*

Recapitulation.

The contents of this paper may be briefly summarized as follows:—

1. The power of curvature is characteristic of organs of such widely different morphological value and physiological function that it cannot be assumed *a priori* that the mechanism of reaction is similar or identical in them all.

2. Two general methods of curvature are known to prevail in various organs of different species. The curvature of stems, petioles, and peduncles, in response to heliotropic and geotropic stimuli, is supposed to be due to elongation of the convex sides of these organs. The movements of pulvini, tentacular hairs of Drosera and the leaves of *Dionaea*, and similar phenomena in other plants, is known to be due to the action of cells on the side becoming convex.

3. Tendrils, irritable to contact, exhibit the greatest difference in morphological derivation, anatomy, and degree of irritability, and it cannot be assumed that their mechanism is identical.

4. The curvature of such highly developed, initially dorsiventral organs as the tendrils of the Passifloreae is due to the contraction of the tissues of the concave side.

5. The curvature of a tendril around a support as a direct reaction to irritation, and the coiling of a free portion of a tendril are entirely distinct, and to a great extent independent processes. The first is due to the action of tissues on the concave side of the organ, the second to the excessive growth of the convex side, and is probably preceded or accompanied by the relaxation of the tissues of the concave side consequent upon loss of irritability.

6. The curvature of a tendril around a support does not accelerate the growth of the convex side. The growth-extension of a coiled portion of a tendril is rarely equal to that of a similar region in a freely nutating organ.

7. The region of maximum growth lies between the middle of a tendril of *Passiflora* and the tip, and never at any time coincides with the region of greatest irritability.

8. The plasmolysis of stimulated or unstimulated tendrils generally results in a shortening of the radius by the contraction of the outer layers of parenchymatous cells of the concave side.

9. The attachment of a tendril to a support influences only in a minor degree the growth and formation of coils of the free portion of the organ. Such influence is due primarily to the traction exerted upon the organ by the weight of the shoot, not to a conduction of the contact stimulus.

10. The amount of strain capable of being exerted during the coiling of the free portion of a tendril is a specific characteristic, and generally is greatly in excess of the weight of the shoot to be supported and drawn upward by the organ. A tendril of *Passiflora* may exert a strain amounting to 10 grams, while that of *Cucurbita* may exert a strain of 30 grams.

11. The strain exerted by a tendril during contact curvature is less than ·5 grams. Rapidity, not force, is essential to the effectiveness of such curves.

12. The time of formation of coils in the free portion of a tendril extends over 6 to 40 hours.

13. The latent period of a contact-reaction varies from 5 seconds to an hour in different species.

14. Marked differences exist between the structure of the protoplasm of the convex and concave sides. The protoplasm of the concave side is more richly granular, and occupies a greater proportion of the cell-cavity than on the convex side. The density of the protoplasm of the convex side increases from the base toward the tip, and apparently corresponds with the degree of irritability to contact exhibited.

15. The parenchymatous cells of the concave side are markedly different in outline, structure, and form from those of the convex side. When plasmolysed they undergo a decrease in size amounting to 20 to 40 per cent. of their original volume, and changes in contour from oblong-ovoid to irregularly globoid or ovoid. During curvature they undergo similar alterations. After curvature they are found in such condition, and to their action must be ascribed the contact-curvatures of the tendrils under examination. This action consists in an increase in the permeability of their protoplasts, a consequent extrusion of water into the intercellular spaces, and a release of the stretching tension exerted upon their walls of the vascular, collenchymatous, and epidermal tissues. The elastic contraction of these cell-walls causes the resultant curvature. This action ensues in free portions of attached tendrils upon maturity, and early in the process of the formation of loose coils in an unattached tendril.

BIBLIOGRAPHY.

I. DARWIN, CH.—Climbing Plants, 1876.

II. DE VRIES.—Längenwachsthum der Ober- und Unterseite krümmender Ranken. Arb. d. Bot. Inst. z. Würzburg, i, 302. 1873.

III. DE VRIES.—Ueber innere Vorgänge bei den Wachsthumskrümmungen mehrzelliger Organe. Bot. Ztg., xxxvii, 835. 1879.

IV. FISCHER, A.—Untersuchungen über das Siebröhrensystem der Cucurbitaceen. Berlin, 1884.

V. GARDINER.—On the power of contractility exhibited by the protoplasm of certain plant-cells. Annals of Botany, i, 362. 1888.

VI. KNIGHT, T. A.—On the motion of tendrils of plants. Phil. Trans. Roy. Soc. London, Part ii, 314. 1812.

VII. KOHL.—Mechanik der Reizkrümmungen. Marburg, 1894.

VIII. KRAUS.—Ueber Wasservertheilung in der Pflanze. II. Der Zellsaft und sein Inhalt. Abh. d. Naturforsch. Ges. z. Halle, xv. 1882.

IX. MACDOUGAL.—The tendrils of Passiflora coerulea. I. Morphology and Anatomy. Bot. Gazette, xvii, 205. 1892.

X. MACDOUGAL.—The tendrils of Passiflora coerulea. II. External phenomena of irritability and coiling. Bot. Gazette, xviii, 123. 1893.

X *a*. MACDOUGAL. Ueber die Mechanik der Windungs- und Krümmungsbewegungen der Ranken. Ber. d. deut. Bot. Ges. xiv, 151. 1896.

XI. MACFARLANE.—Contributions to the history of Dionaea muscipula. Cont. Bot. Lab. Univ. of Penn., i, 7. 1892.

XII. MASTERS.—Contributions to the natural history of the Passifloraceae. Trans. Linn. Soc., 593. 1870.

XIII. MOHL.—Ranken und Schlingpflanzen. 1827.

XIV. MÜLLER, O.—Untersuch. über die Ranken der Cucurbitaceen, Cohn's Beiträge zur Biologie der Pflanzen, iv, 97. 1887.

XV. NOLL.—Beitrag zur Kenntniss der physikalischen Vorgänge die den Reizkrümmungen zu Grunde liegen. Arb. d. Bot. Inst. z. Würzburg, iii. 496. 1888.

XVI. NOLL.—Ueber die Mechanik der Krümmungsbewegungen der Pflanzen. Flora, viii, 36. 1895.

XVII. PENHALLOW.—Mechanism of Movement in Cucurbita, Vitis, and Robinia. Proc. Roy. Soc. of Canada, iv, sec. 4, p. 49. 1886.

XVIII. PFEFFER.—Zur Kenntniss der Kontaktreize. Untersuch. a. d. Bot. Inst. z. Tübingen, i, 497. 1885.

XIX. RUSSELL.—Recherches sur la vrille des Passiflorés. Bull. Soc. d. France, xxvii, 189. 1890.

XX. SACHS.—Physiology of Plants. Eng. Ed., p. 665. 1887.

XXI. WIESNER.—Die heliotropischen Erscheinungen im Pflanzenreich. II. Theil. 1880.

XXII. WORGITZKY.—Vergleichende Anatomie der Ranken. Flora, lxx, 2, 17, 33, 49, 65, 86. 1887.

XXIII. WORTMANN.—Zur Kenntniss der Reizbewegungen. Bot. Ztg. xlv, 785, 801, 817, 833. 1887.

XXIV. WORTMANN.—Einige weitere Versuche über die Reizbewegungen vielzelliger Organe. Ber. d. deut. Bot. Ges., v, 459. 1887.

XXV. WORTMANN.—Zur Beurtheilung der Krümmungserscheinungen der Pflanzen. Bot. Ztg., xlvi, 469, 485. 1888.

XXVI. WORTMANN.—Beitrag zur Physiologie des Wachsthums. Bot. Ztg., xlvii, 229, 245, 261, 277, 293. 1889.

XXVII. WORTMANN.—Ueber die Beziehungen der Reizbewegungen wachsender Organe zu den normalen Wachsthumserscheinungen. Bot. Ztg., xlvii, 453, 468. 1889.

EXPLANATION OF FIGURES IN PLATE XIX.

Illustrating Prof. MacDougal's paper on the Mechanism of Tendrils.

All the figures were made freehand from longitudinal sections of tendrils of *Passiflora coerulea*, and with the exception of Fig. 5, include the epidermis, collenchyma, and cortical parenchyma.

Fig. I. Showing size, arrangement, and contour of cortical parenchyma-cells of concave side in free coils. Prepared from fresh material. × 350.

Fig. II. Showing tissues of convex side in free coil. Prepared from fresh material. ×350.

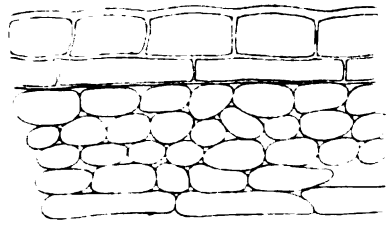
Fig. III. Tissues of concave side of active tendril before stimulation. Killed with acetic-alcohol and imbedded in paraffin. ×550.

Fig. IV. Showing tissue of convex side of active tendril before stimulation. Killed with acetic-alcohol, and imbedded in paraffin. ×350.

Fig. V. Infolded end-walls of pith. Prepared from fresh material. ×300.

Fig. VI. Showing contour of tissues of concave side after curvature. Killed in acetic-alcohol and imbedded in paraffin. ×350.

Fig. VII. Tissues of convex side after curvature. Killed in acetic-alcohol and imbedded in paraffin. ×350.



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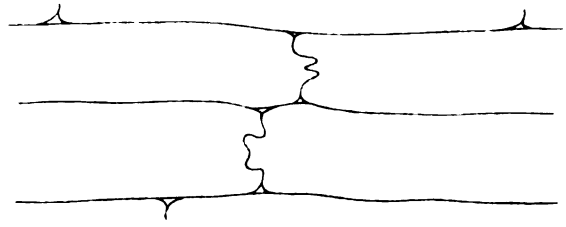

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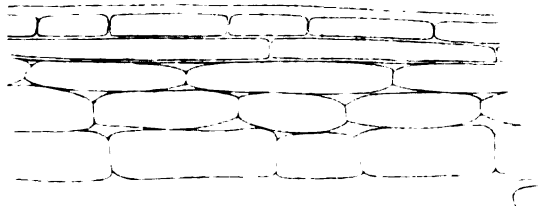

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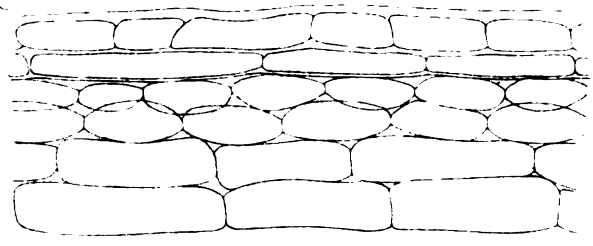

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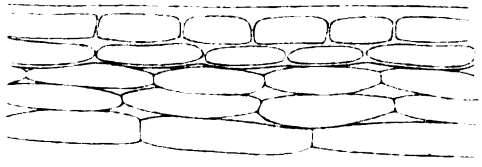

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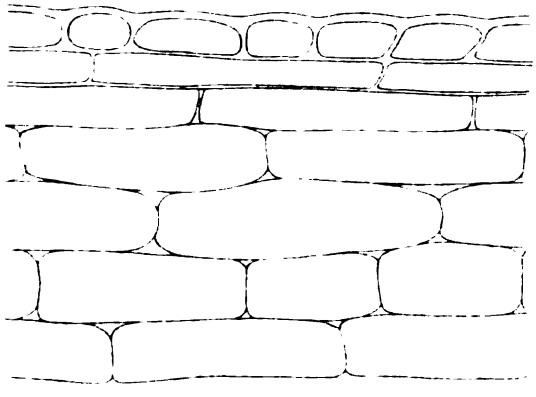

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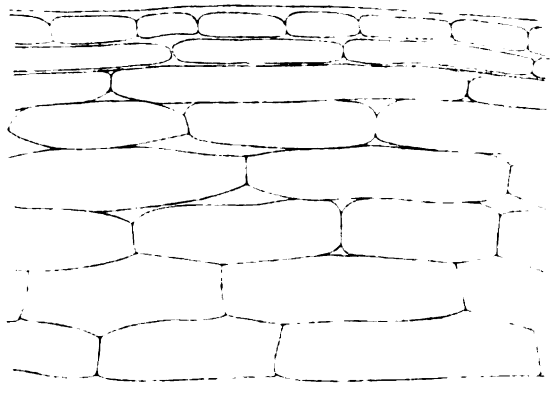
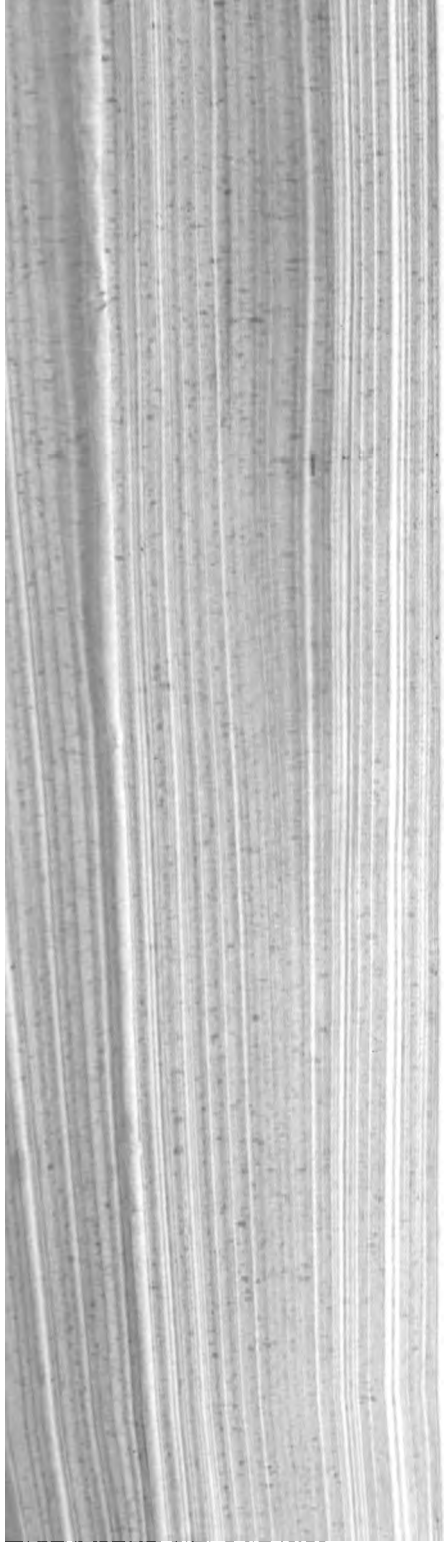


VII

J. Tilden & J. S. Wright del. University Press, Oxford

MAC DOUGAL. — TENDRILS.



On the Life-History of Rhabdonia tenera, J. Ag.

BY

WINTHROP J. V. OSTERHOUT.

With Plates XX and XXI.

RHABDONIA TENERA, J. Ag., is a characteristic species of the warmer waters of the eastern coast of the United States, and is found abundantly in sheltered bays and coves. The abundance of the plant in certain localities near Woods Hole, Mass., together with the fact that certain peculiarities in the structure and development of the cystocarp and the proliferation of the tetrasporic plant seemed to be little understood, led the writer, while studying at the Marine Biological Laboratory at Woods Hole, to begin the investigation of the life-history of this plant with the hope of throwing some light upon these and other points.

The species was first described by J. G. Agardh ('41) as *Gigartina tenera*, from specimens from the West Indies. Later ('51) he referred it to Harvey's genus *Rhabdonia*. Harvey ('52) described it anew, and referred it to the European *Solieria chordalis*. J. G. Agardh ('71) subsequently stated that Harvey's plant is his *Rhabdonia tenera*. Farlow ('75 and '81) has given the best account of the cystocarp. Schmitz ('89) separated the species from the genus *Rhabdonia*, and placed it in a new genus *Agardhiella* belonging to the Cystoclonieae. As the description of the new genus has not yet appeared, I have preferred to retain the older name.

[Annals of Botany, Vol. X. No. XXXIX. September, 1896.]

Methods.

The best results were obtained from material which was fixed in a saturated solution of picric acid in sea-water, washed fifteen to twenty hours in running sea-water and preserved in sea-water, to which was added plenty of camphor broken up into small pieces. Material so treated is still in excellent condition after standing for over a year. Material killed in chromic acid (1 % in sea-water), or picric acid and preserved in formalin (2 % in sea-water), is also very satisfactory, while material placed directly in 2 % formalin without previous treatment retains its colour for some time and is kept in excellent condition. The addition of 1 % chrome-alum to the formalin seems to be an advantage.

At the commencement of the work hand-sections were made with a razor; but as it soon became evident that some more efficient method of sectioning was necessary, the material was embedded in paraffin and serial sections were cut. The shrivelling of the tissue in all except very young parts of the frond was so great that this method was abandoned. Freezing by means of ether was then tried, and excellent results were obtained; but this method of freezing proved so troublesome, that in looking about for a better one, the writer began experimenting with ice and salt, with the result that an apparatus was finally devised which could be operated with very little trouble and which proved very efficient, a description of which appeared in the Botanical Gazette for May, 1896 (vol. xxi. p. 195). It was found that large frozen sections when cut very thin, easily broke in pieces in the region of the medulla. To avoid this the material was placed before sectioning in 50 % gelatine[1] for a short time;

[1] This is prepared as follows: The best gold-label gelatine is carefully brushed clean and used according to the following formula:—

Gelatine	100 grm.
Distilled water . . .	100 c.c.
Thymol	Trace.

Mix over a water-bath, and add the white of one egg to clarify.

the gelatine was then removed from the exterior by washing in warm water, and the object was placed on the freezing chamber in a gum-arabic solution and cut. Most excellent results were obtained by this method, and all delicate parts were held in place. The sections were removed from the knife, placed in a watch-glass in 20 % glycerine, which was allowed to stand until it became considerably more concentrated; enough 50 % gelatine was then added over a water-bath to make a good glycerine-jelly, and the sections were placed on a slide in a drop of this and mounted. The whole process can be carried out on the slide, but it is much easier to treat a great many sections at once in a watch-glass. If it is not desirable to mount them at once, it is only necessary to protect the glycerine-jelly from evaporation, and it can be kept indefinitely. In many cases it was found convenient to prepare thick sections in this way, and mount them between two covers so that they could be studied from both sides. If it is desirable to fix such a preparation permanently to the slide, Canada-balsam makes a sufficiently good cement.

Sections made from material not previously infiltrated with gelatine were mounted in glycerine-jelly or glycerine. In the latter case it was found advantageous to mount in 20 % glycerine, and after the latter had become more concentrated by standing, to ring the preparation with a syrupy solution of gum-arabic. This forms around the edge of the cover a glycerine-gum which becomes hard in a short time, and the preparation may then be ringed with Canada-balsam.

Numerous stains were tried, of which Heidenhain's iron-alum haematoxylin proved to be by far the best. Anilin stains and the ordinary haematoxylin mixtures have the disadvantage that they stain the gelatinous wall and intercellular substance deeply. On the other hand a purely aqueous haematoxylin and Schneider's aceto-carmin are free from this objection, but they stain all protoplasmic structures rather diffusely. With Heidenhain's method the gelatinous

wall and intercellular substance, as well as the cytoplasm, can be washed out so as to be entirely unstained, while the nucleolus, chromatin, linin, chromatophore, and the refractive plates accompanying the pits stain dark blue or black. Material stained *in toto* may be subsequently boiled, and employed in making crushed preparations without impairing the stain. Such material should remain from one to several hours in the haematoxylin.

External Anatomy.

The plants are often two to three feet long, varying from a light yellowish red to deep red, cylindrical, tapering at the base, and attached by a discoid holdfast. The branches are numerous, alternately decompound, long and straight, and tapering at the base and apex. The antheridia, cystocarps, and tetraspores are borne on distinct plants. The three kinds of plants resemble each other closely, but the cystocarpic plants are as a rule more robust, and are easily distinguished by the prominent swellings on the branches, which are produced by the immersed cystocarps projecting on one side of the branch. The older tetrasporic plants bear on their older portions numerous short, simple proliferations, which bristle out from the frond in all directions. The antheridial plants resemble the tetrasporic, but lack the proliferations.

Histology.

The cells at the growing-point are small and closely crowded together, which makes a study of their arrangement rather difficult. By clearing slightly with potash, and by contracting the cell-contents with glycerine, one can make out in most cases a central filament of cells, which gives off on all sides lateral branches which grow obliquely upwards, making an acute angle with the central filament. Fig. 9 shows the

central filament *c*, *c*, *c*, &c., and the lateral branches. It is not always possible to decide whether there is one or several central filaments. A careful study, however, of the tips of ordinary branches, and especially of the proliferations on the tetrasporic plants, strongly favours the conclusion that only one such filament is present. Each lateral branch ramifies so as to produce a corymbose cluster of branchlets; these clusters are firmly held together by a common gelatinous intercellular substance, so as to constitute a firm parenchyma-like tissue. Fig. 10 shows a section passing through two such clusters.

Cell-division is almost exclusively confined to the terminal cells of the branches and their branchlets; the frond therefore grows only at the surface. The terminal cells divide longitudinally, transversely, or obliquely, or they cut off from the distal end segments on all sides by means of oblique walls. The method of branching is in consequence very irregular. The vegetative growth of the thallus conforms to the rule laid down by Schmitz ('83 and '92), according to which the Florideae possess thalli which are composed of branching filaments, and only the terminal cells of these filaments ever divide transversely or in such a way that the dividing-wall lies in the organic long axis of the cell. Exceptions to this rule are found in the Corallinaceae (Schmitz, '92), in the Delesseriaceae (Schmitz, '92; Naegeli und Schwendener, '77; Kny, '79; and Reinke, '80), and in the cells which form the pericarp in *Rhabdonia*. In all these cases the same individual shows a transition, in some or all of its parts, from a filamentous to a non-filamentous mode of growth. This transition forms an interesting parallel to the similar development which must have taken place in the Chlorophyceae, if they represent the ancestral stock from which the Liverworts are descended.

A median longitudinal section of the frond passing through the apex, shows that the cells of the central filament, as well as those which surround it, become more and more elongated the further they are removed from the apex. At the same time they tend to assume a direction parallel to the long

axis of the frond. At a short distance from the growing-point it is impossible to distinguish the central filament from the cells which surround it. The cells occupying the interior of the frond finally become long, narrow tubes, much curved and bent, resembling those of the medulla of *Fucus*. The highly refractive plates between the cells become developed to an unusual degree (Fig. 10 at the left). In this manner a medulla arises composed of a varying number of tubular longitudinal filaments, which anastomose laterally in an irregular manner, and lie imbedded in a gelatinous substance which fills the central portion of the frond. The medulla traverses the frond throughout its entire length.

The cells lying externally to the medulla constitute the cortex. Those forming the exterior of the frond are relatively small and elongated in the radial direction (Fig. 10). They are densely filled with protoplasm, and have large, deeply-coloured chromatophores. They form the most actively assimilating layer. Passing inward towards the medulla, the cells of the cortex grow rapidly larger, becoming rounded or ellipsoid. At the same time they grow poorer in protoplasmic contents, and the chromatophores become paler and more reduced the nearer the cells lie to the medulla.

Physiologically considered, the relations between the cells are not unlike those of a radial leaf, especially one with a poorly-developed central-bundle system. The actively assimilating outermost layer, composed of cells directed perpendicularly to the surface, corresponds to the palisade-layer. The rounded cortical cells have large intercellular spaces filled with water containing the necessary gases for the metabolism of the innermost cells of the interior, much as the spongy parenchyma provides for the circulation of gases in the leaf. The inner cortical cells also serve to convey elaborated products to the elongated cells of the medulla, which probably convey them to the growing-point and also to the holdfast, where they are stored up as Floridean starch. Wille ('85) mentions *Rhabdonia* as having

a mechanical system with the mechanical elements arranged in a ring around the conducting system. The numerous types into which he has sought to separate the Florideae on anatomical-physiological grounds have so little value, that I have not considered it necessary to refer *Rhabdonia* to any one of them. In his work on the storage-system of Florideae (Wille, '87), *Rhabdonia* is not mentioned.

The structure in the region of the holdfast corresponds in general to that described above, but the intercellular spaces are almost entirely lacking, and the closely packed cells, which are arranged so as to give the greatest mechanical strength, are square or angular in section. Just above the holdfast the distinction between medulla and cortex entirely disappears. All the cells of the holdfast and the region directly above it are densely filled with Floridean starch. It is not impossible that in winter the plant may die down to the holdfast, which may in the spring regenerate the lost portions.

The cortical cells near the tip of the frond often bear long unicellular hairs, which soon drop off and leave the surface of the frond perfectly smooth.

The cells are connected by primary pits which arise at division, and by secondary ones which are formed subsequently. Shortly after the formation of a pit, the cell-wall immediately around it becomes swollen and highly refractive; and, as the cell grows older, forms a fairly thick, highly refractive circular plate (Fig. 10 at the left). In the oldest cells it often happens that such plates become separated into two, which are connected by a delicate strand of apparently protoplasmic substance (Fig. 1, between *st* and *cpg*).

If the connecting substance is really protoplasm, it is without doubt continuous through the plates with the protoplasm of the cells lying on either side. According to Wille ('85) such plates in *Cystoclonium purpurascens* and some other Florideae are pierced by numerous pores like the callus-plates of Laminariaceae.

Both kinds of spores are uninucleate. The younger

vegetative cells are as a rule uninucleate, while the older ones are multinucleate. The nuclei contain fairly large nucleoli with a coarse linin-network, on which the chromatin is arranged in coarse, scattered granules.

The chromatophore in the youngest cells is a plate which almost entirely covers the wall on the inside. As the cells increase in size, the chromatophore becomes band-shaped, with long, irregular serpentine branches, which wander about over the inner surface of the cell-wall, and occasionally anastomose (Fig. 16). In both kinds of spores the chromatophores are of this character. The cells in the interior of the oldest portion of the frond gradually lose all trace of chromatophore and become colourless. Starch-grains, which turn reddish-brown and finally violet with iodine, are abundant in the cells in the region of the holdfast, but in other parts of the frond they seem to be entirely lacking.

Antheridia.

The antheridia, which have not been hitherto described, occur on all parts of the male plants, forming larger or smaller patches or even a continuous covering extending over considerable portions of the plant. They originate from the outermost cells of the cortex, which by oblique divisions cut off at the distal end small cells often to the number of four or five (Fig. 16). Each of these cells then cuts off a single small cell by a transverse wall, or two small cells by oblique walls. These either become antheridia, or divide in the same way, and bear the antheridia in their turn. The antheridia are very small elongated cells filled with clear, colourless protoplasm, which is sometimes more or less granular and vacuolate. They are borne either singly or in twos; in the latter case they stand side by side. The escaped antherozoid is a small, slightly elongated, naked mass of clear protoplasm.

Structure of Mature Cystocarp.

The cystocarps are immersed in the frond, which they cause to swell out on one side in a striking manner. A cross-section of the frond passing through the centre of the cystocarp shows that just outside the latter the cortex is thicker, and composed of smaller cells than it is elsewhere, and that an opening, the carpostome, leads from the outside to the cystocarp. The central part of the latter, commonly called the 'placenta,' is spherical in shape, and is composed of sterile cells which have no chromatophores. They are very irregular in size and shape. Surrounding this sterile mass is a sporiferous layer composed of radiating filaments of spores from two to five cells in length, and often bearing two to four-celled branches. The sporiferous tissue is interrupted here and there by strands of elongated sterile cells proceeding from the 'placenta' to a fairly thick pericarp, which surrounds the spores, and is composed of radiating filaments of cells which are smaller than those of the 'placenta' and elongated in the tangential direction. This elongation is especially marked in the outermost cells, which come to resemble the cells of the medulla with which they form secondary connexions by means of pits.

The pericarp forms a complete investment to the cystocarp, except at the carpostome, and the application of reagents brings out the fact that it is enclosed in a special gelatinous envelope, which separates it from the medulla on which it abuts.

In the older cystocarps spores are often found which divide irregularly and produce rhizoids, but their development is apparently soon arrested.

Development of the Procarp.

Procarps originate as a rule very close to the growing tips of the frond, but sometimes a few scattered procarps are formed in somewhat older portions of the plant, where the majority of the procarps are ready for fertilization. They

arise as follows: one of the lateral branches composing the cortex cuts off, usually midway between the medulla and the surface, a single cell which is situated on the upper side of the branch. This cell divides in a plane parallel to the long axis of the branch which bears it, and the uppermost cell divides in its turn, so that a three-celled branch is formed, which is directed obliquely upward and inward (Fig. 10). Occasionally four- and five-celled procarpic branches are found. The development of the trichogyne begins with the formation of a small papilla filled with protoplasm, which is clear and free from granules (Fig. 15). It elongates slightly, and then recurves abruptly on the cell which bears it, the trichophore, so that the tip is directed towards the surface of the frond (Fig. 10). It grows directly outward towards this, occasionally making a more or less complete spiral turn, and finally reaches the surface and protrudes through the gelatinous covering of the frond (Fig. 11). In the meantime it has elongated considerably at the base, so that the point at which it recurves is further from the trichophore than it was originally (Figs. 10 and 11). The whole trichogyne is at this time filled with clear protoplasm nearly free from granules, and with few, if any, vacuoles. The wall is very delicate, and is surrounded by a gelatinous layer, often as thick as the trichogyne (Fig. 11). During its growth the trichogyne has a more or less swollen tip, but when it reaches the surface the tip appears no longer swollen but of about the same diameter as the rest of the trichogyne and slightly tapering.

The protoplasm of the trichophore, which is at first free from granules and vacuoles, acquires vacuoles as the trichogyne develops, and becomes more or less granular. The cells of the procarpic branch below the trichophore contain reduced chromatophores with sometimes a mere trace of colour, but are usually without any colour whatever. Their protoplasm very early becomes vacuolate and granular. Occasionally one of them (in the majority of cases the one next the trichophore) bears a lateral cell.

Fusion of the Antherozoid.

Only a few cases of the fusion of the antherozoid were found, but in all of these the fusion occurred very near the tip of the trichogyne. . Wherever trichogynes could be traced with certainty to the surface, they did not project much beyond the gelatinous coating of the frond (Fig. 11). Trichogynes with attached antherozoids were found only in crushed preparations in which it was not possible to determine how far the trichogyne projected beyond the jelly. From the facts observed, it seems probable that the trichogyne does not project very much beyond the jelly, though this point is one of some uncertainty.

Conjugating-tube.

After fertilization, the contents of the trichophore lose all connexion with the contents of the trichogyne, being separated from them (Fig. 13) by a considerable interval filled by the gelatinous wall which encroaches on the lumen of the trichogyne, and finally completely fills it. The trichophore then puts out on one side a short projection filled with clear protoplasm, which grows into a long, delicate tube, tapering gradually towards the end (Fig. 13, *ct*). The contents of the tube are slightly granular and at first free from vacuoles, but these gradually make their appearance, being as a rule small and scattered. The tube grows towards the tip of the frond, keeping in the medulla and avoiding the cortex. Shortly after the tube begins to grow out from the trichophore, a second (Fig. 13), and often a third, makes its appearance, and grows in the same general direction as the first. It is less common to find only a single tube proceeding from the trichophore, but such cases are not difficult to find. These tubes, to which the name 'conjugating-tubes' will be applied, often reach a surprising length, and become very much attenuated towards the end. Occasionally a tube is found with one or more globose or ovoid swellings somewhere in its continuity. At a point opposite to an auxiliary cell, the

tube bends outwards and makes its way through the cortex to the auxiliary cell. As the conjugating-tube develops, the trichogyne is displaced to one side and gradually becomes disorganized, the disorganization proceeding from the tip towards the point of attachment (Fig. 13 *tr*).

Origin of the Auxiliary Cells.

Certain lateral branches of the cortex begin very early to have a different appearance from the ordinary branches which surround them on all sides. Their cells become more densely filled with granular protoplasm, and in many of them the chromatophore becomes paler. The basal cell of every such branch rests on one of the elongated cells of the medulla, is usually much elongated and has less protoplasmic contents than the other cells of the branch (Fig. 2, *a*). The next cell is larger (Fig. 2, *st*) and has fairly dense contents, and frequently bears one or more lateral branches. The next cell of the branch is commonly somewhat constricted in the middle; this third cell in the branch is the auxiliary cell (Fig. 2, *cpg*) with which the conjugating-tube later unites. The auxiliary cell always bears three large cells, one at the top (Fig. 2, *b*), and two just below it on the outer side (Fig. 2, *c* and *d*). The auxiliary cell, together with the three cells which are borne on it, and the cell upon which it rests, form a group of five large cells, which possess colourless chromatophores, and are densely filled with granular protoplasm. Each of the three cells borne upon the auxiliary cell gives rise to several branches which branch more or less freely, and form a mass of small cells lying externally to and around the auxiliary cell (Figs. 3, 4, 5 and 1).

These possess for the most part well-developed chromatophores, which are deeply coloured as those of other cortical cells of the same size which are borne on purely vegetative branches, but the first-mentioned cells are distinguished from the last-mentioned by their position and by their richer protoplasmic contents.

Fusion with the Auxiliary Cell.

The conjugating-tube, which proceeds from the trichophore, appears in all cases to unite with the auxiliary cell near its base (Figs. 12 and 14); at the point of union the conjugating-tube is more or less swollen and closely pressed against the auxiliary cell. The wall between them is absorbed, and the contents of the conjugating-tube unite with those of the auxiliary cell (Fig. 14).

The number of auxiliary cells is very small compared with the number of procarps, not over two per cent.; consequently they are quite certain to be found by some of the numerous conjugating-tubes.

Segmentation of the Carpogenic Cell.

After conjugation the auxiliary cell cuts off a segment by an oblique wall near the top and on its inner side (Fig. 3). The cell thus cut off divides and gives rise to a mass of comparatively small cells densely filled with protoplasm (Figs. 4 and 5). By growth and repeated division the mass enlarges and finally completely surrounds the auxiliary cell, and also the three large cells which are borne upon it (Fig. 1). It is then possible to see that the whole mass is made up of radiating filaments of cells, the gonimoblastic filaments, which branch in a somewhat irregular manner, and at the ends bear short branched filaments of small cells, densely filled with protoplasm, which become spores (Fig. 1). Thus there arises an irregularly spherical mass of cells composed of gonimoblastic filaments radiating in all directions from the carpogenic cell (Fig. 1. *cpg*) and bearing spores at the surface of the mass, the interior of which (the 'placenta') is composed of sterile cells which have enlarged considerably and lost most of their contents, with the exception of a delicate film of protoplasm lining the cell-wall. Imbedded in this mass are the three cells which were mentioned before as being borne on the auxiliary cell, and which are now very large and almost entirely destitute of protoplasm.

Many of the terminal cells of the gonimoblastic filaments which radiate from the carpogenic cell, instead of changing into spores, grow out into sterile filaments, often branching dichotomously, and composed of a few elongated cells (Fig. 1, *x*, *x*, *x*, &c.) These reach the pericarp and attach themselves to it, forming connexions by means of secondary pits with the cells of its innermost layer.

Development of the Pericarp.

The sterile cells of the branch containing the auxiliary cell give off branching filaments of cells, and perhaps a few such filaments occasionally proceed from the auxiliary cell; these divide very irregularly, not following the rule for the division of ordinary vegetative cells, and by the time that conjugation with the auxiliary cell takes place, they form a pericarp investing on all sides the auxiliary cell and its four large accompanying cells, already mentioned (Figs. 2, 3, 4, and 5). In that portion of the pericarp lying towards the interior of the frond the divisions take place in such a way that numerous rows of cells are formed, radiating from the centre of the mass, which is occupied by the auxiliary cell and its accompanying cells (Fig. 4). Nearest the centre of the mass the cells of each filament are small, and there is a regular increase in the size of the cells going towards the periphery; this is accompanied by an elongation of the cells in the tangential direction. In the case of the outermost ones this elongation is so great that they come to resemble the elongated cells of the medulla of the frond (Figs. 4 and 1). The portion of the pericarp lying outside the auxiliary cell forms the cortex of the frond at that point (Figs. 2–5). It is composed of branching filaments like the ordinary cortex, but the cells are from the first more densely filled with granular protoplasm. These cells begin, shortly after conjugation with the auxiliary cell takes place, to grow and divide rapidly, forming a mass which, as compared with the ordinary cortex, is much thicker and composed of smaller cells. The entire pericarp thus develops almost

exclusively from the sterile cells of the branch containing the auxiliary cell.

Development of the Carpostome.

The development of the carpostome begins quite early, and by the time conjugation with the auxiliary cell has taken place a considerable depression has been formed in the exterior of the frond just over the auxiliary cell (Fig. 3). It arises as follows: some of the filaments proceeding from one of the two lateral cells borne by the auxiliary cell cease to grow, and finally the terminal cells (i. e. the outermost) perish (Figs. 2, 3, 4, 5 and 1). These cells are easily recognized in preparations of living material by their greenish colour. The cells of the filament then die as far back as the large cell which is borne on the auxiliary cell, and an opening is thus made to the exterior. In the meantime the filaments in the immediate neighbourhood have commenced the rapid growth already mentioned, and those nearest the carpostome produce around it a lining of very small cells (Fig. 1). A surface view of the carpostome shows the cortical cells of the pericarp arranged in rows, which radiate from the carpostome in all directions.

Theoretical Considerations.

Whether the cystocarp of the Florideae represents a sporophyte or not, must remain for the present an open question. The fact that we are not able to say whether the trichophore or the auxiliary cell, or both, are fertilized does not affect the question, so long as the sexuality itself is not called in question. The grounds on which the sexuality of the Florideae has been questioned, are the result of erroneous or incomplete observation. The statements of Bornet and Thuret to the effect that the cystocarp often originates from cells which are separated from the trichophore and which do not fuse with it, were made under the impression that fusion was not necessary to ensure fertilization. They consequently

gave the matter of fusion no special attention. These statements have been corrected by later investigation. Davis ('96) has recently questioned the sexuality of *Batrachospermum*, but my own observations, not yet published, so far from confirming his results, show conclusively that a true fertilization takes place. Wille ('94) has also been able to demonstrate fertilization in *Nemalion*. Until further and conclusive evidence to the contrary has been brought forward, we must assume that the cystocarp is the result of a sexual act. But, as De Bary ('70) long ago pointed out, the cell from which it originates does not become morphologically separate from the mother-plant. In Mosses, Ferns and Flowering-plants, the sporophyte-generation begins with a cell which is morphologically separate from the gametophyte on which it is borne. Too much stress need not be laid on morphological separation, since in the Phanerogams the mother-cell of the embryo-sac, which is generally assumed to be the starting-point of the female gametophyte-generation, shows no more morphological independence than the trichophore of the Florideae. The question cannot be decided until it has been determined at what point in the life-cycle the reduction to one half the number of chromosomes takes place. The general behaviour of the cystocarp points to the conclusion that an alternation of generations is really present.

Whether this be really the case or not, it is interesting to notice how the development of the cystocarp finds a striking parallel in that of the sporophyte of Liverworts. In its most elementary form it consists almost entirely of sporogenous tissue (*Callithamnion*) like the sporocarp of *Riccia*. In its most highly developed form it consists of more or less sporogenous tissue borne on and nourished by a mass of sterile tissue (the 'placenta' of *Rhabdonia*), corresponding to the foot and columella of *Anthoceros*. Between these extremes numerous intermediate forms are present in both groups. Positive proof of the homology of the two structures is at present lacking, but the analogy, if it be nothing more, is most striking.

It is interesting to note how the Liverworts, responding to terrestrial conditions, have in the higher forms raised the sporophyte above the thallus to secure a better dissemination of the spores, while in the Florideae the opposite tendency has been manifested; protection has been the determining factor, and the cystocarp has, in the higher forms, been buried beneath the surface of the frond much as the sporophyte is in the lower Hepaticae. The formation of a protective covering by the sporophyte is peculiar to the Liverworts; and, while not a character of fundamental importance, shows a higher degree of development.

TETRASPORANGIA.

The tetrasporangia develop directly from the outermost cells of the cortex (i. e. from apical cells of the cortical filaments). They become enlarged, and densely filled with protoplasm; the nucleus divides into two and then into four; the protoplasm by 'free cell-formation' separates into four masses somewhat rounded off from each other, but not separated by cell-walls. In this condition they remain until they escape from the tetrasporangium or commence to germinate in position.

The tetrasporangium, during its growth, encroaches on the surrounding cells, and distorts them; when mature it often has a double wall (Fig. 6), the inner layer of which is thinner and somewhat denser than the outer. The tetraspores escape by the rupture of the gelatinous wall of the sporangium; they remain for a short time enclosed in a delicate membrane; this is then dissolved, and the spores separate from each other, become spherical, and finally become attached to the substratum and germinate. The germination proceeds exactly like that of the carpospores, the first walls being laid down without any regular arrangement.

GERMINATION OF TETRASPORES.

The older tetrasporic plants commonly bear on the older portions of the plant numerous short simple proliferations,

which are not found on the male or female plants. They often reach a length of an inch and a half, and bristle out from the frond in all directions. These proliferations are found to originate from tetrasporangia, which germinate inside the frond, the whole contents of a sporangium taking part in the formation of each proliferation. The process begins with the division of all the spores by means of oblique walls which do not occur in regular succession (Fig. 7). For a time it is possible to see distinctly the four separate masses of cells which result from the process, but as the process continues the boundaries between the originally distinct masses become obliterated, and one finds only a mass of cells occupying the place of the sporangium and preserving its general outline (Fig. 8), but already encroaching decidedly on the surrounding cells, and also projecting from the frond. The mass grows most rapidly in that portion lying nearest the outside of the frond, from which it gradually grows out to form the proliferations just described. As it does so the cells of the tip begin to show a definite arrangement into a central filament, which bears numerous short appressed branching filaments (Fig. 9), the terminal cells of which form the exterior of the frond. In the meantime the cells at the base of the proliferation grow out into rhizoidal filaments (Fig. 9), which make their way between the cortical cells of the parent frond, and finally reach the elongated cells of the medulla and form secondary connexions with them.

Some of the proliferations remain sterile, but in the majority of cases they bear reproductive organs often before they are more than a quarter of an inch in length. Antheridial proliferations are very common, while tetrasporic and cystocarpic ones are rather rare; but the three kinds often occur growing side by side.

The union of several spores to form a single individual is by no means an isolated phenomenon, though it cannot be said to be a common one. Proliferations resembling those of *Rhabdonia* occur on the tetrasporic plants of *Cystoclonium*,

Gracilaria, and other higher Florideae, and it is probable that they originate in a similar way.

According to De Bary ('84, p. 2) the germinating spores of *Nectria Solani* unite (in groups of from two to eight) by means of short tubes to form a single individual. Woronin ('95) describes a similar case in connexion with the germinating spores of *Sclerotinia Padi* and *S. Aucupariae*. These cases are homologous with that of *Rhabdonia*, if we consider that the tetraspores which are contained in the same sporangium are morphologically independent. This seems to be the case, since they are capable under favourable circumstances of separating from each other and germinating independently without having in the meantime undergone any modification other than that of escaping from the sporangium. When they remain enclosed in the mother-plant, they undergo a most fundamental modification, in that each spore, instead of developing into a separate individual, produces only a small portion of one, and so perfect is the correlation that the outermost spore produces merely a growing-point, whilst the innermost develops rhizoids. Whether the case in question belongs to the category of strictly epigenetic phenomena or is an inherited character, I do not attempt to decide.

Two views are easily possible as to the physiological conditions obtaining previous to germination. It may be assumed that physiological and morphological independence are acquired at the same time, and that the former is gradually lost as the result of the fact that the spores are unable to escape from the sporangium and separate. In the case of abnormal eggs of *Ascaris*, described by Strassen ('96), two eggs remain in contact by means of a broad protoplasmic connexion, and throw off the first polar body independently; but they unite to make a second polar body common to both eggs. Later they develop into a single embryo of unusual size. The separate individualities are apparently gradually merged into one as the result of continued union; it must, however, be remembered that the

case in question is an abnormality of rare occurrence, which has not been thoroughly investigated, and that it is moreover quite susceptible of a different interpretation. The above-mentioned cases of *Nectria* and *Sclerotinia* show that spores may fuse and give up their individuality completely, but these are exceptional cases. In the majority of cases individuality once established is not subsequently surrendered as the result of fusion (apart from the case of gametes), as is shown by the conidia of Ustilagineae, which after fusing put out separate germ-tubes exactly as though no fusion had occurred. On the whole, therefore, the assumption that physiological independence is first established and subsequently lost, rests on no direct evidence, and finds its chief justification in the general consideration that physiological and morphological independence usually go hand in hand.

On the other hand it is possible to assume that the spores contained in a single sporangium probably form a physiological unit so long as they remain in contact, or at least that they do not acquire complete physiological independence. The whole contents of the sporangium are so polarized that at one end they produce a growing-point, at the other end rhizoids which penetrate to the medulla of the mother-plant. The polarity of the individual spores is not developed, or at any rate does not manifest itself, until they become separated from each other so as to germinate separately, which, as stated above, not unfrequently happens. The separate spores may be compared to a magnet composed of four separate pieces placed end to end. As long as they remain in contact there are but two principal poles for the whole, but as soon as they are separated, each is polarized independently. Or they may be compared physiologically to an animal egg in the four-cell stage, each of whose cells if separated from the others produces an embryo. A still better comparison is furnished by the embryo-like structure in Dicyemidae, which develops from an egg, but at a certain stage of its development falls into separate cells, each of which (with the exception of one)

develops into an embryo (Whitman, '83). On this view, the case of *Rhabdonia*, though homologous with that of *Nectria* and *Sclerotinia*, is not analogous with them.

The purpose of this peculiar method of development is much easier to understand in the case of *Sclerotinia* than in *Rhabdonia*. The spores of the former germinate on the stigma of the host-plant, and, according to Woronin, are unable, when they germinate independently, to produce a germ-tube long enough to traverse the entire length of the style and reach the ovary. By uniting they are able to produce a much more vigorous germ-tube, which reaches the ovary with no difficulty. In the case of *Rhabdonia* the object seems to be entirely different: the plants produced by the union of four spores remain extremely small in comparison with those which in all probability are produced from a single spore. Here the object is perhaps to produce a large number of antheridia (the proliferations most commonly bear antheridia), and so to compensate for the small number of antheridial plants.

In the case of *Nectria* it seems natural to suppose that the union of several spores produces a plant which at the start has an advantage in competition with those plants which are produced from a single spore. This is probably also the case in the Ustilagineae. It is doubtless of advantage in the Florideae, and certainly seems to be so in *Sclerotinia*. Exactly what the nature of this advantage is, is not clear, but it may be that it claims a distant kinship with that which results from the fusion of two gametes, and perhaps affords a hint as to the mode of origin of the sexual act.

RESULTS.

The frond is composed of branching filaments which grow by division of the terminal cells. At the growing-point there is a single central filament which gives off branches on all sides.

The procarp is usually a three-celled branch borne on one

of the lateral branches of the cortex. The terminal cell of the branch produces an abruptly recurved trichogyne, which makes its way to the surface, and the antherozoid unites with it at the tip. The trichogyne then loses its connexion with the trichophore, and the latter puts out one or two conjugating-tubes which make their way through the medulla toward the tip of the frond and unite with the auxiliary cells. The latter are cells in the continuity of certain specialized lateral branches of the cortex; each specialized branch contains a single auxiliary cell; the remaining cells of the branch (with the exception of the basal one) develop sterile filaments which surround the auxiliary cell and form the pericarp. The auxiliary cell, after the conjugating-tube reaches and conjugates with it, gives rise to a mass of radiating filaments which bear at the ends short branching filaments of cells which become the spores. The basal portions of the radiating filaments remain sterile and constitute the placenta. Some of the filaments grow out into chains of sterile cells which reach the pericarp and connect the placenta with it. In the meantime a carpostome has been formed by the disorganization of some of the filaments lying externally to the cystocarp.

The occurrence of numerous short proliferations on the tetrasporic plant is known in several of the larger Florideae, and naturally excites some interest. In the case of *Rhabdonia* these proliferations are independent plants, arising from the germination of tetraspores in position in the parent plant on which they occur. A point of interest is that it is not a single tetraspore, but the whole contents of a tetrasporangium, which is required to produce one of these plants.

The formation at the base of the young plant of rhizoids which make their way to the medulla of the parent plant and form protoplasmic connexions with its cells, suggests that the young plants are probably partly parasitic on the parent.

Another point of interest is the occurrence on these young plants, often before they are more than a quarter of an inch in length, of antheridia (very commonly), and occasionally of

tetraspores and cystocarps. Two kinds of reproductive organs never occur on the same proliferation.

In conclusion, I wish to express my most sincere thanks to Prof. W. A. Setchell, at whose suggestion the work was undertaken, and whose direction and assistance have been of the greatest value throughout its progress.

BIBLIOGRAPHY.

AGARDH, J. G., '41: Linnaea. Vol. xv, p. 18. 1841.

——— ——— '51: Species, Genera et Ordines Algarum. Vol. ii, p. 354. 1851.

——— ——— '71: Bidrag til Florideernes Systematik, Acta Univ. Lundensis. Vol. viii, p. 38. 1871.

DE BARY, A., '70: Beiträge zur Morph. und Phys. der Pilze, von A. de Bary und M. Woronin. Dritte Reihe, p. 86. Frankfurt, 1870.

——— ——— '84: Vergl. Morph. und Biol. der Pilze, Mycetozoen und Bakterien. Leipzig, 1884.

DAVIS, B. M., '96: The fertilization of *Batrachospermum*. Annals of Bot. Vol. x, p. 49. March, 1896.

FARLOW, W. G., '75: Proc. Amer. Acad. Vol. x, p. 368. 1875.

——— ——— '81: Marine Algae of New England, p. 159, Pl. 14, Fig. 2. 1881.

HARVEY, W. H., '52: Nereis Bor.—Amer., part II, p. 120, Pl. 33*a*. 1852.

KNY, L., '79: Bot. Wandtafeln, Abth. III, Erläuternder Text, p. 81. 1879.

NAEGELI UND SCHWENDENER, '77: Das Mikroskop, 2. Auflage, pp. 562–563. 1877.

REINKE, '80: Lehrbuch der Botanik, p. 116 ff. Figs. 77 and 78. 1880.

SCHMITZ, Fr., '83: Sitzungsberichte der Berliner Akad. der Wissenschaften. 1883.

——— ——— '89: Systematische Uebersicht der bisher bekannten Gattungen der Florideen. Flora, Bd. XLVII, p. 441. 1889.

——— ——— '92: La Nuova Notarisia, pp. 111–114. 1892.

ZUR STRASSEN, O., '96: Riesenembryonen bei *Ascaris*. Biol. Cent. XVI, pp. 426–431. 1896.

WHITMAN, C. O. Contributions to the life-history of the Dicyemids. Mittheilungen aus der zool. Station zu Neapel, Bd. IV, 1883.

WILLE, N., '85: Bidrag til Algernes Physiologiske Anatomi. Kongl. Svenska Vetenskaps-Akademiens Handlingar. Bandet XXI, No. 12. 1885.

——— ——— '87: Beiträge zur Entwickelungsgeschichte der physiologischen Gewebesysteme bei einigen Florideen. Nova Acta der Ksl. Leop.-Carol. Deutschen Akad. der Naturforscher, Bd. LII, Nr. 2. 1887.

——— ——— '94: Bericht über die Verhandlungen der 11. Gen.-Vers. der Deutschen Bot. Ges. p. 57. 1894.

WORONIN, '95. Die Sclerotienkrankheit der gemeinen Traubkirsche und der Eberesche, Mém. de l'Acad. Imp. des Sci. de St. Pétersbourg. VIII, Sér., vol. 2, No. 1, 1895.

EXPLANATION OF FIGURES IN PLATES XX AND XXI.

Illustrating Mr. Osterhout's paper on the Life-History of *Rhabdonia tenera*, J. Ag.

All the drawings were made from nature by the author with the aid of the Abbé camera.

Figs. 1–5 are from material killed in picric acid and preserved in camphor water, subsequently infiltrated with gelatine and cut when frozen.

Fig. 1. Median longitudinal section of a young cystocarp: *cpg.*, carpogenic cell (i.e. auxiliary cell); *cs.*, carpostome; *st.*, stalk-cell; *b* and *c*, accompanying cells; *ee* &c., *ff* &c., *gg* &c., *hh* &c., *ii* &c., gonimoblastic filaments proceeding from the carpogenic cell and bearing spores at the ends. The spores are shaded in the figure: x, x, x, &c., sterile filaments connecting the placenta with the pericarp. × 196.

Fig. 2. Median longitudinal section of frond showing auxiliary cell, *cpg*, before the conjugating-tube has united with it. *a*, basal cell of branch; other letters as in preceding figure. The carpostome, *cs*, has just commenced to develop, as is indicated by the external depression in the frond. × 274.

Fig. 3. Median longitudinal section of frond. The auxiliary cell, *cpg*, has cut off a small cell (shaded in the figure) which has divided into four. Another, a sister-cell, which lies above it and which has not yet divided, is not shown in the figure. The carpostome, *cs*, is further developed than in the preceding figure. The cells external to the cell *h* (which lies under the cells immediately surrounding it in the figure) have died away, causing a depression in the surface of the frond. The pericarp consists of a few radiating filaments of cells. Letters as in Fig. 1. × 384.

Fig. 4. Median longitudinal section of frond. The small cells which are shaded in Fig. 3 have begun to grow out into gonimoblastic filaments which here consist of a mass of small crowded cells (shaded in the figure). The carpostome, *cs*, and pericarp are further developed than in the preceding figure. Letters as in Fig. 1. × 384.

Fig. 5. Median longitudinal section of frond. The gonimoblastic filaments (shaded in the figure) begin to show their filamentous character. The carpostome, *cs*, has the position and shape which it possesses in the mature cystocarp. Letters as in Fig. 1. × 384.

Figures 6–9 are drawn from living material.

Fig. 6. Transverse section of frond, showing mature tetrasporangium with a thinner inner wall and a thicker outer one. The transverse lines in the tetrasporangium indicate the surfaces of contact of the spores, which are masses of protoplasm not separated by cell-walls. × 280.

Fig. 7. Transverse section of frond, showing the spores dividing inside the tetrasporangium. × 280.

Fig. 8. More advanced stage of same; the lines of separation between the four masses of cells resulting from the division of the tetraspores are no longer visible. × 280.

Fig. 9. More advanced stage than Fig. 8. The tip of the young plant is seen in median optical section, showing a central filament (*c*, *c*, *c* &c.) and the branches which proceed from it. At the base of the plant rhizoidal filaments, *rh*, are beginning to grow out. ×280.

Fig. 10. Median longitudinal section of frond, showing the elongated cells of the medulla with highly refractive plates between the cells. At the right, two lateral branches of the cortex; from the lower arises a young procarp. ×420.

Fig. 11. Median longitudinal section of frond, showing trichogyne reaching surface. ×474.

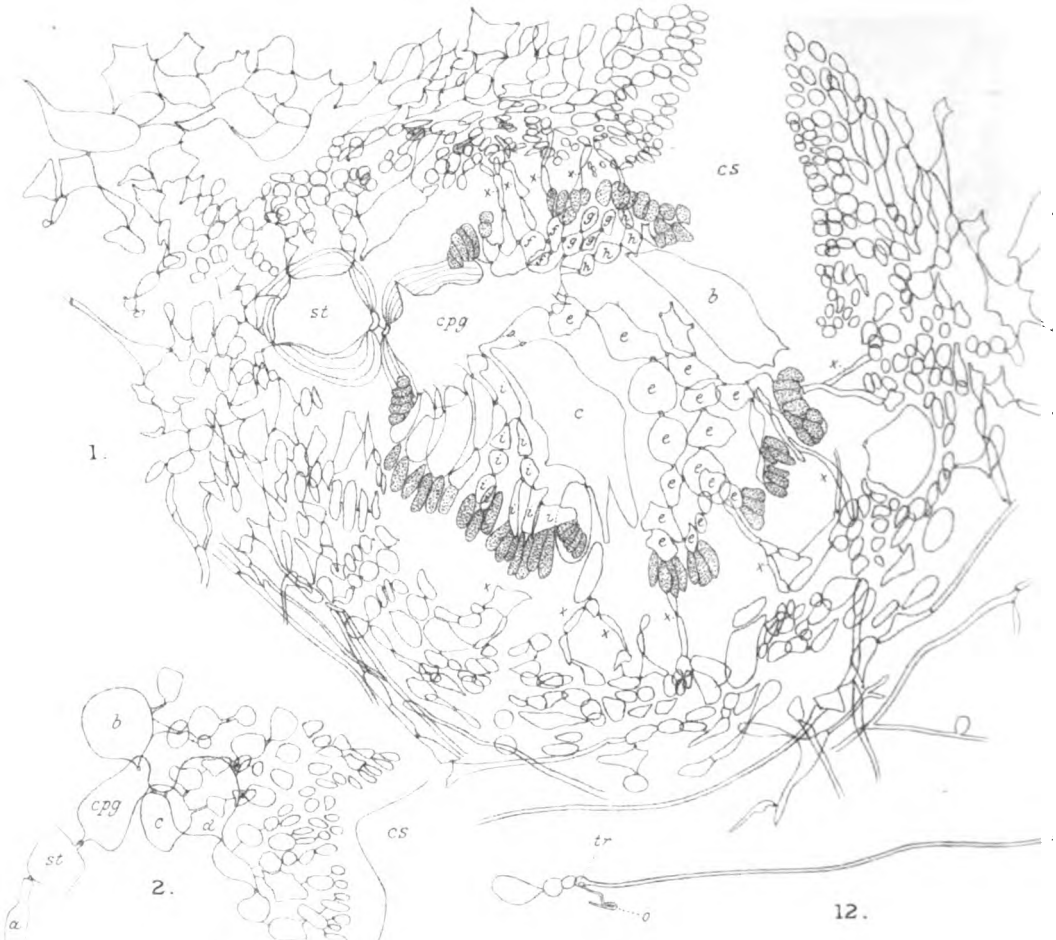
Fig. 12. Trichophore, *tr*, putting out a conjugating-tube which has reached an auxiliary cell, *cpg*, and conjugated with it. *o*, a second (abortive?) conjugating-tube. Other letters as in Fig. 2. From a crushed preparation. ×128.

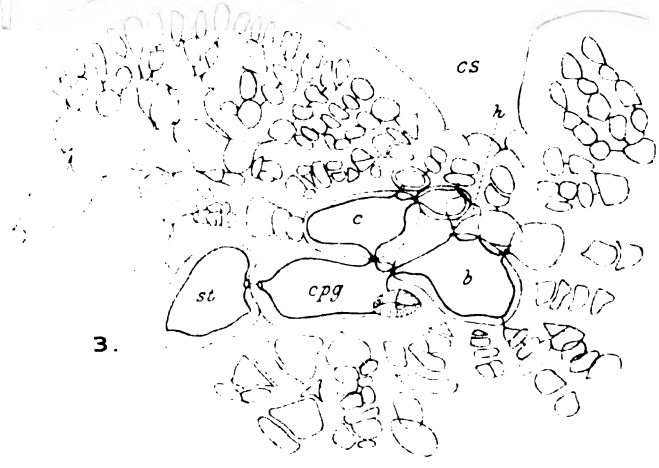
Fig. 13. Trichophore sending out two conjugating-tubes *ct*; the trichogyne, *tr*, is pushed to one side and is disintegrating at the end. From crushed preparation. ×535.

Fig. 14. Part of Fig. 12 enlarged; letters as in Fig. 12. The conjugating-tube, *ct*, proceeding from the trichophore shown in Fig. 12, *tr*, is seen conjugating with the auxiliary cell. From crushed preparation. ×550.

Fig. 15. Procarp showing the beginning of a trichogyne. From crushed preparation. ×770.

Fig. 16. Transverse section of male plant, showing antheridia. From living material. ×1160.





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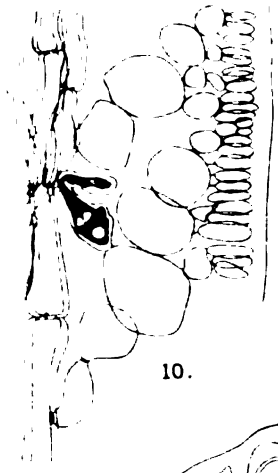

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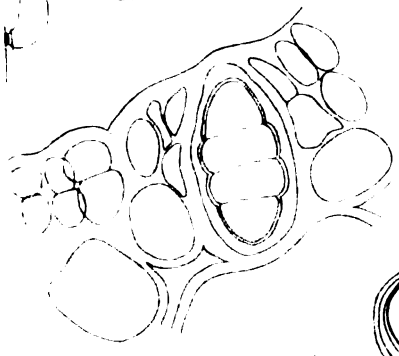

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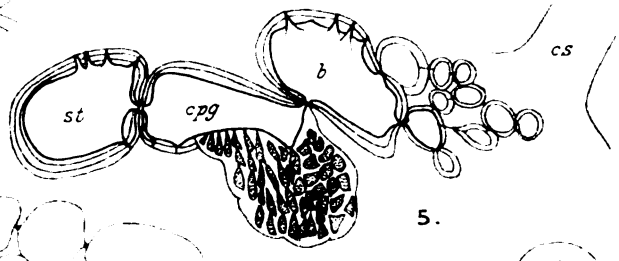

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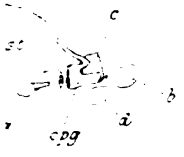

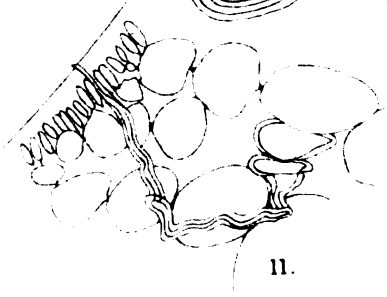

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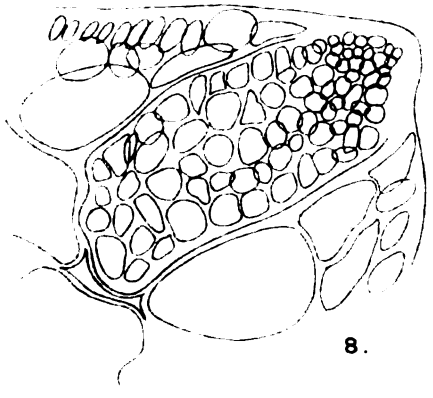

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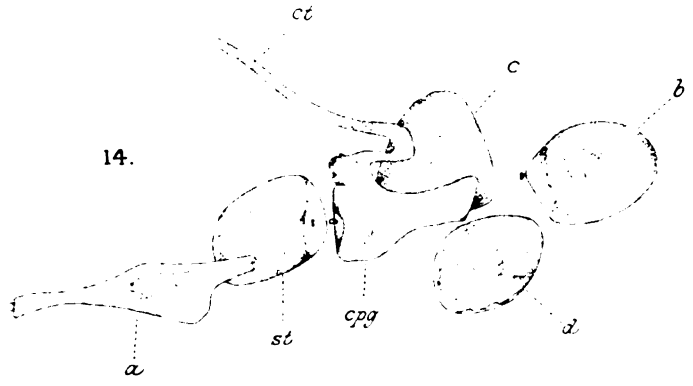

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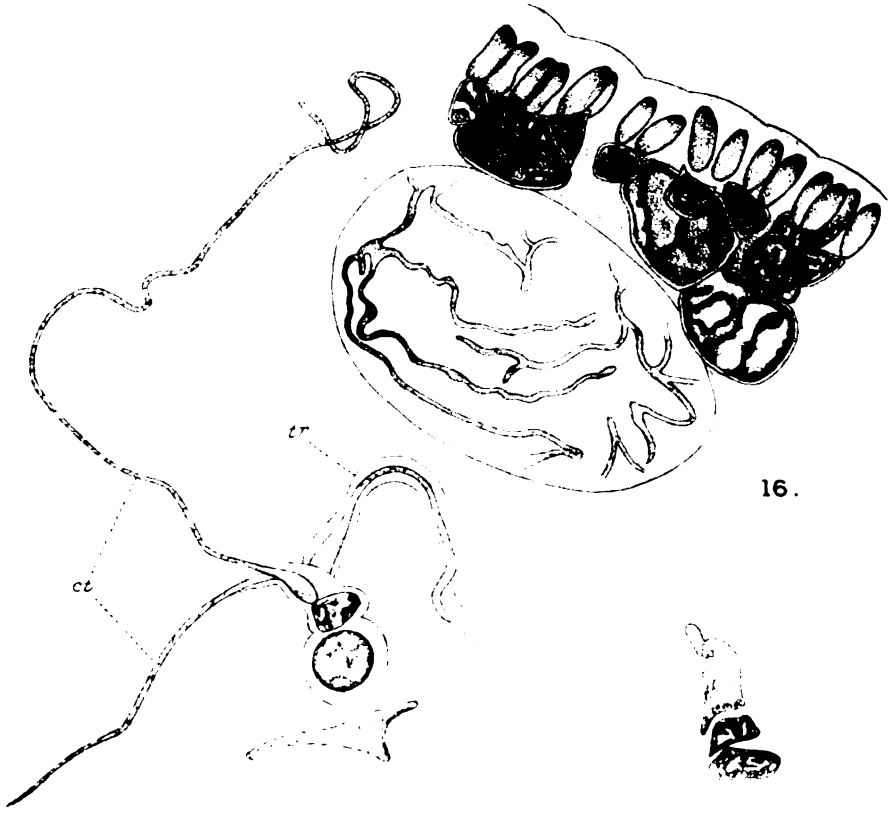

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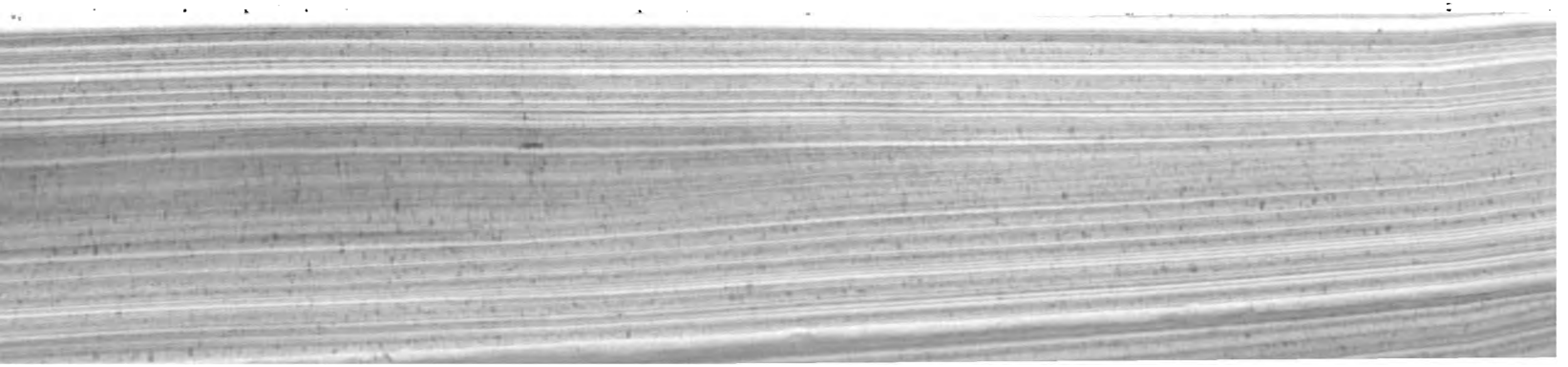


The Suction-force of Transpiring Branches [1].

BY

S. H. VINES.

OF all the still incompletely solved problems of the physiology of plants, none has received so much attention of recent years as that of the means by which a current of water is maintained in a lofty tree between the absorbing roots on the one hand, and the transpiring leaves on the other. Without entering upon a discussion of all the various theories which have been proposed to account for this fact, it may be pointed out briefly that, according to prevalent opinion, it cannot be satisfactorily explained on the imbibition-theory of von Sachs [2], nor on the gas-pressure theories of Hartig [3] and Boehm [4], nor on the vitalistic theory of Godlewski [5]; and that it is not merely a phenomenon of capillarity. It would appear, from the researches of Dixon and Joly [6] and of Askenasy [7], that the motive power is the suction-force of

[1] I have published a brief note on this subject in the last (June, 1896) number of the Annals, p. 292.

[2] Vorlesungen ueb. Pflanzenphysiologie, 2te Aufl., 1887, p. 221. (Eng. Ed., p. 241.)

[3] Die Gasdrucktheorie, 1883.

[4] Warum steigt der Saft in den Bäumen, Wien, 1878; also Ann. d. sci. nat., sér. 6, t. 6, 1878.

[5] Pringsheim's Jahrbücher, XV, 1884.

[6] Annals of Botany, Vol. viii, 1894; Proc. Roy. Soc., Vol. lvii, 1895; Phil. Trans., 1895.

[7] Verhandl. d. naturhist.-med. Vereins zu Heidelberg, Neue Folge, Bd. v, 1895 and 1896.

[Annals of Botany, Vol. X. No. XXXIX. September, 1896.]

the transpiring surfaces. The former make the following definite statement of their conclusion [1]:—'Our theory is that this (the suction-force, *Saugkraft*, of the leaf) is the all-sufficient cause of the elevation of the sap, not however by establishing differences of gas-pressure, but by exerting a simple tensile stress on the liquid in the conduits': and Askenasy says [2],—'the warmth of the sun causes evaporation at the external surface of the mesophyll-cells, the imbibition-force of the wall of these cells sucks water from their contents, thus increasing their osmotic capacity: this gives rise to a pull which, in virtue of the cohesion of the water (we assume for the moment the existence of continuous columns of water in the conducting tissues, which is certainly true in many cases), is propagated to the root, and thus affects the living cells of the root: here it is reconverted into osmotic force which promotes absorption by the roots, provided that they are in contact with water.'

Such being the current views on the subject, it becomes a matter of importance to study and measure this suction-force; to determine the relation of the force to the transpiring leaf-area and to variations in external conditions; and to analyse it into its ultimate physical factors.

Experiments of this sort are by no means new. So long ago as 1726, Hales [3] instituted 'experiments whereby to find out the force with which trees imbibe moisture'; and the same has been done since by Meyen [4], Unger [5], von Sachs [6], von Höhnel [7], Boehm [8], and others. In all cases the method of experimentation has been essentially the same. A cut-off branch was attached air-tight to a glass-tube filled with water,

[1] Phil. Trans., p. 563. [2] Loc. cit., 1895, p. 11.

[3] Statical Essays, Vol. i, 4th ed., 1769, p. 84.

[4] System der Pflanzenphysiologie, 1838, Vol. ii, p. 70.

[5] Beiträge zur Anatomie und Physiologie der Pflanzen, XIII; in Sitzungsber. d. k. Akad. d. Wiss., Wien, Vol. 50, 1864.

[6] Experimental-Physiologie, 1865, p. 260.

[7] Ueb. den negativen Druck der Gefässluft, Inaug. Diss., Wien, 1876.

[8] Ber. d. deut. bot. Ges., VIII, 1889; Bot. Centralblatt, 42, 1890; Ber. d. deut. bot. Ges., XI, 1893.

the other end of the tube dipping into an open vessel containing mercury: as the water at the upper part of the tube was absorbed and transpired by the branch, the mercury rose in the tube from below.

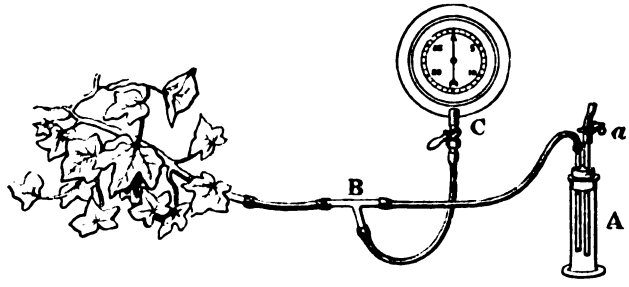
But it is clear that an experiment thus arranged is not well adapted to demonstrate and estimate the suction-force generated in connexion with transpiration: for it is obvious that the rise of the mercury in the tube, up to a height of thirty inches (760 mm.) or so, is not the result of a tensile stress exerted by the transpiring branch on the liquid in the tube, but is due to the atmospheric pressure acting upon the mercury in the open vessel. It is surprising how small were the results obtained by this method. Thus Hales observed, with an apple-branch two feet high, a rise of twelve inches of mercury in the tube: Meyen, with a shoot of *Vicia Faba*, seven inches in four hours: Unger, with a shoot of *Eupatorium cannabinum*, a rise of 110 mm.: von Sachs, with a small branch of *Aesculus Hippocastanum*, a rise of 20 mm. in nine hours; and with a Cabbage-leaf, a rise of 30 mm. in twenty-four hours: von Höhnel, with a small branch of *Syringa vulgaris*, a rise of 300 mm. Boehm alone appears to have succeeded in observing a rise of the mercury in the tube above the height attributable to the atmospheric pressure: in his most recent (1893) account of his work, he mentions two experiments in which this occurred, both being made with branches of *Thuja*: in the one the mercury rose to a height of 864 mm., with the barometer at 751 mm.; in the other it rose to 906 mm., with the barometer at 745 mm. Hence, in the one case, the suction-force of the branch alone was (864–751) 113 mm.; in the other it was (906–745) 161 mm.

This mode of experimentation has the disadvantage that the conditions under which the water is absorbed by the branch differ materially from those obtaining in nature, in that the atmospheric pressure intervenes; whereas in the uninjured plant the atmospheric pressure does not directly affect the absorption and transmission of water by the wood which constitutes an air-tight system. It occurred to me that

a mode of experimentation in which the conditions should more nearly approach the normal, would be one in which the water in contact with the cut-off branch was enclosed in an air-tight vessel, thus excluding the intervention of the atmospheric pressure. Moreover, in such a case, any measurements which might be obtained would have reference solely to the suction-force of the branch. In the first experiments, made last year, the air-tight tube, entirely filled with water, to which the branch or shoot was attached, communicated with a mercurial barometer as a means of measurement, the fall of the mercury in the barometer indicating the suction-force of the branch. By this means I obtained values for the suction-force, which were much higher than any I have met with recorded elsewhere. In these experiments I used the Cherry-Laurel (*Prunus Laurocerasus*) as representing woody plants, and the Sunflower (*Helianthus annuus*) as representing herbaceous plants: with the former I obtained as a maximum a fall of 320 mm. of the mercury in the barometer-tube, and with the latter a fall of 116 mm. I cannot, however, attach great importance to these results, as the experiments were few in number, for they had to be brought to an abrupt conclusion.

This spring I resumed experiments on the subject, but with an important alteration in the apparatus, namely this, that for the mercurial barometer I substituted a Bourdon's vacuum-gauge. The apparatus, as used, is represented in the accompanying Woodcut 1, where a branch is attached to an india-rubber tube connected with one arm of a T-tube at *B*, the two other arms of which are respectively connected by india-rubber tubes with the gauge *C*, and with a glass tube leading into the water-reservoir *A*. The water-reservoir is closed by an india-rubber stopper, through which pass two glass tubes, the one of which is connected with the T-tube, whilst the other carries a small piece of india-rubber tubing at its free end, and can therefore be hermetically closed by means of a pinch-cock at *a*. In preparing for an experiment the branch is attached to its india-rubber tube under such

conditions that the cut surface is maintained in contact with water: in some cases I removed the cortex from the portion of the branch introduced into the india-rubber tube, in others I simply attached the branch as it was, without any perceptible difference in behaviour. When all the connexions are made, the tubing is filled with water by raising and inverting the water-reservoir *A*; then the reservoir itself is completely filled by raising the tube *a* so that its lower end does not project beyond the lower surface of the india-rubber stopper, and then pouring water down it from a wash-bottle, when all the air escapes up the tube: the tube *a* is then lowered so that its lower end is about on a level with that of the other tube



Woodcut 1.

in the reservoir; and, finally, the india-rubber tube attached to its free end is closed at *a* by two stop-cocks whilst still full of water. The apparatus, if properly set up, is now entirely free from gas; it is air-tight, and the indicator of the vacuum-gauge stands at zero. It should be mentioned that the india-rubber tubing must be provided with an internal spiral wire to give it strength to withstand the atmospheric pressure from without, so as not to become occluded; or, better still, it should be thick-walled pressure-tubing; and the water used must have been boiled, so as to render it as nearly as possible air-free. The dial of the gauge is divided into thirty parts, representing inches of mercury, so that a complete revolution of the indicator would mark a perfect vacuum.

As the branch begins to exert stress on the water in the apparatus, the indicator moves round the dial, marking the degree of tension to which the water in the tubes is being subjected.

I have found, like my predecessors, that gas is evolved, sooner or later, from the cut end of the branch into the tubing. This does not occur, however, until the gauge indicates considerable tension: for instance, in the case of the Beech-branches, at tensions varying from 5–21 in.; and in the case of *Helianthus*, at a tension of about 5 in. But in this apparatus, the evolution of bubbles of gas from the branch does not affect the indicator of the gauge, provided that the gas is not allowed to accumulate between the branch and the T-piece so as to interrupt the continuity of the water in the tube. The evolution of gas by branches freshly cut from the tree is frequently so rapid that I found it impossible to keep the tube clear: consequently I was obliged to abandon freshly cut branches of Beech, and to use only such as had been kept standing in water for twenty-four to forty-eight hours after having been cut. The Yew gives off gas much less readily, so that in this case freshly cut branches might be used: one such branch did not begin to give off bubbles until a tension of over 17¼ in. had been reached: another similar branch which had been kept standing in water for forty-eight hours after having been cut from the tree, did not give off bubbles until a tension 19¼ in. had been attained: moreover the volume of gas evolved was small in both cases.

If at any time during an experiment a leakage develops in the tubing so that air enters, the indicator goes backward on the dial; and if the leakage is considerable, or if the stop-cock be opened, the indicator at once returns to zero, and the water sinks in the tube *a*.

I may now pass to an account of some illustrative experiments, beginning with the Beech (*Fagus sylvatica*) and the Yew (*Taxus baccata*), as representing woody perennial plants. The branches used were cut from large trees. The experiments were carried on in a well-lighted room facing south:

lay in a horizontal position on a table close to a

Beech-branch (*A*) with about 1,150 leaves: diameter of cut end = ⅛ in.: standing 24 hours in water: rather hot, sunny:—

1.20 p.m.	indicator	= 0 in.	1.50 p.m.	indicator	= 17 in.
1.25 ,,	,,	= 6¾ ,,	2 ,,	,,	= 18¼ ,,
1.30 ,,	,,	= 11¾ ,,	2.10 ,,	,,	= 19¼ ,,
1.33 ,,	(first bubble)	= 13 ,,	2.20 ,,	,,	= 20 ,,
1.40 ,,	,,	= 15¾ ,,			

Readjusted apparatus, removing gas and refilling with water:—

2.40 p.m.	indicator	= 0 in.	3.10 p.m.	indicator	= 20½ in.
2.50 ,,	,,	= 14 ,,	3.20 ,,	,,	= 20¾ ,,
2.55 ,,	(first bubble)	= 16¾ ,,	3.35 ,,	,,	= 21¼ ,,
3 ,,	,,	= 18½ ,,	3.40 ,,	,,	= 21¼ ,,

July 1.—Branch of the Yew: freshly cut: about 3 ft. 6 in. long: diameter of cut end = ½ in.: weather cloudy, temp. 64° F.:—

July 1.			July 2.		
1.15 p.m.	indicator	= 0 in.	Gas accumulated in tube during the night; gas removed.		
1.25 ,,	,,	= 1 ,,			
2.30 ,,	,,	= 13¼ ,,	9 a.m.	indicator	= 22 in.
3.30 ,,	,,	= 15½ ,,	10 ,,	,,	= 22 ,,
4.30 ,,	,,	= 16¾ ,,	10.30 ,,	,,	= 22¼ ,,
5 ,,	,,	= 17¼ ,,	11 ,,	,,	= 22¼ ,,

The following is the record of an experiment with the Sunflower (*Helianthus annuus*) as representing herbaceous plants:—

June 8.—Pot-plant 2 ft. high, with 13 leaves of various sizes: kept in dark room and well watered for 40 hours previously to the experiment: stem cut off under water: the remaining stump shows strong root-pressure: weather hot, sunny; plant began to flag visibly at 11.45 a.m.:—

11 a.m.	indicator	= 0 in.	1.30 p.m.	(bubbles)	= 5¼ in.
11.15 ,,	,,	= ½ ,,	2 ,,	indicator	= 6¼ ,,
11.30 ,,	,,	= 1¼ ,,	3 ,,	,,	= 7½ ,,
Noon	,,	= 2½ ,,	4 ,,	,,	= 9½ ,,
12.30 p.m.	,,	= 3½ ,,	5 ,,	,,	= 11 ,,
1 p.m.	,,	= 4½ ,,			

The question now arises as to the significance of such figures as these; what is it that they represent? This question may be met with the reply that they represent the

suction-force of the branch under experiment. But this is no real answer, for the further question at once arises, what is the nature of this force?—can it be definitely shown to be of the nature of a tensile stress; or is it merely a measure of the tension of the gases in the wood, a measure, that is, of the negative pressure developed in the branch?

The first step towards a solution of this somewhat difficult problem is to obtain a clear idea of what is going on in the branch whilst the experiment is in progress. It must be understood that, so long as no gas-bubbles are evolved from its cut surface, the shoot is not absorbing any water from the apparatus. If any direct proof of this obvious physical fact is necessary, it is afforded by the rapid flagging of the leaves and younger parts of the shoots of *Helianthus* when under experiment: their cells very soon lose their turgidity, in consequence, no doubt, of transpiration uncompensated by absorption. When bubbles are evolved, however, each bubble displaces a corresponding volume of water from the apparatus, so that water is then absorbed by the shoot; in an experiment with an actively transpiring branch, a considerable proportion ($\frac{1}{3}$–$\frac{1}{2}$) of the water in the reservoir may eventually be replaced by gases evolved from the cut end of the stem.

These are the actual facts; but how are they to be explained? What is the active cause of the escape of bubbles of gas from the cut end of the stem? This gas cannot be air leaking through the shoot, for were it so there would necessarily be a fall of the tension indicated on the dial: it is gas derived from the tissue of the stem, some of it, possibly, escaping out of solution in the water of the tissues. Various explanations of the escape of this gas might be suggested: but as the matter is a purely physical one, I will not attempt to discuss it in detail. For my own part, I incline to the view that the escape of bubbles is due to a stress exerted upon the water in the tube by the suction-force of the branch, with the result that water is absorbed and gas expelled in corresponding volume; and that, consequently, the tension indicated on the dial is not a measure of the tension of the gases (negative

pressure) in the branch, but is a measure of a tensile stress exerted by the branch.

The escape of bubbles of gas from the cut surface of the shoot into the apparatus does not, at the moment, affect the position of the indicator on the dial, because the gas is at the same tension as the water in the apparatus: but as the gas evolved at lower tensions is removed from the tubes and accumulates in the reservoir *A*, it will gradually expand as the tension increases, and thus lowers the absolute measurements of the tension obtained.

A point of interest is the consideration of the different tensions at which gas-bubbles begin to escape from the cut surfaces of the shoots. In the case of the Beech I have the following tensions recorded:—13, 16¾, 21¼, 16, 5, 9¾, 13½ inches; and in the case of *Helianthus* 5–7½ in. The tension at which bubbles begin to escape would seem to be determined by the degree of tension of the fluids in the shoot at the beginning of the experiment; for I found that the tension was relatively high when the shoot had been previously well supplied with water, and relatively low when this was not the case. For instance (June 18), a branch of Beech which had been standing in darkness with its cut end in water for twenty-seven hours began to evolve bubbles at a tension of 5 in.: but after remaining all night connected with a tube by which water was injected into its cut surface by a slight siphon-action, it did not evolve bubbles next morning (June 19) until the tension reached 9¾ in. In most cases no gas was evolved by shoots from which all the leaves had been removed, in consequence, probably, of the low tensions attained.

The highest tensions which I have observed are 23 inches of mercury with the Beech, and 23¾ in. with the Yew, tensions which would raise water to a height of about 24–25 feet, in the case of relatively small shoots. This important fact being established, it becomes necessary to distinguish the factors which co-operate in developing so considerable a force.

In the first place, the enquiry may be made as to the relation between the bulk of a shoot, as a whole, and its suction-force; and for the purposes of such an enquiry the following data, referring to four different branches of Beech, are available :—

Branch.	*A.*	*B.*	*C.*	*D.*
Length of main shoot . . .	7 ft.	5 ft.	6 ft.	5 ft.
Diameter of cut surface . .	$\frac{5}{8}$ in.	$\frac{3}{8}$ in.	$\frac{1}{2}$ in.	$\frac{1}{2}$ in.
Number of leaves . . .	1150	320	755	594
Maximum tension observed . .	$21\frac{1}{2}$ in.	$14\frac{1}{4}$ in.	$16\frac{1}{4}$ in.	23 in.
Gas-bubbles evolved at tension of	$21\frac{1}{4}$ in.	11 in.	11 in.	12 in.

Now the 'bulk' of a shoot includes the important factor of the leaf-area, a factor which might well be thought to be preponderant. But a consideration of the foregoing figures shows that no definite relation can be traced between the leaf-area of the four branches and their suction-force. This remarkable fact comes out even more strikingly when the leaf-area of one and the same branch is diminished at intervals by the removal of some of the leaves, as follows (the letters refer to the branches already mentioned):—

Shoot A.

Time.		*No. of leaves.*	*Indicator travelled.*	*No. of leaves removed.*
June 5.	11.10–noon . .	1150	0–$21\frac{1}{2}$ in. max.	(330)
	12.10–1.5 p.m. . .	820	0–20 ,, ,,	(270)
	1.20–2.35 . . .	550	0–$18\frac{1}{2}$,, ,,	(195)
	2.45–3.40 . . .	355	0–20 ,, ,,	(110)
	3.50–5.40 . . .	245	0–$18\frac{1}{4}$,, ,,	
June 6.	10.35 a.m.–12.50 p.m.	245	0–$20\frac{3}{4}$,, ,,	(245)
	1.10–3.50 p.m. . .	leafless stem	0–$4\frac{3}{4}$,, ,,	

Shoot B.

June 9.	1–4.30 p.m. . .	320	0–$13\frac{3}{4}$,, ,,	(81)
,, 11.	11.45 a.m.–6.15 p.m. .	239	0–$9\frac{1}{2}$,, ,,	(94)
,, 12.	12.15 p.m.–5 p.m. .	145	$8\frac{1}{2}$–$12\frac{1}{4}$,, ,,	(102)
,, 15.	3.50 p.m.–5 p.m. .	43	0–$5\frac{1}{2}$,, ,,	

Shoot C.

June 19.	10.15–11.45 a.m.	.	755	0–15½ in.	(400)
	12–12.40 p.m. .	.	355	0–16¾ ,,	(212)
	12.45–1.30 p.m. .	.	143	0–15½ ,,	(82)
	1.50–5.20 ,, .	.	61	0–16¼ ,,	(61)
	5.35 p.m. .	.	indicator	= 16¼ ,,	
June 20.	3.5–9.45 p.m. .	.	leafless stem	= 0–8½ ,,	

Shoot D.

June 24.	1 p.m.–5 p.m. .	.	594	0–23 in.	(250)
,, 25.	9.50 a.m.–4 p.m.	.	344	0–18½ ,,	
	4.30 p.m. . .	.	indicator	= 18½ ,,	
	4.45 ,, . .	.	,,	= 18½ ,,	

The bulk of the branches themselves, as distinguished from the leaves which they bear, seems to have some relation to the suction-force of a shoot. For instance, a shoot (*C*) of Beech, from which all the leaves had been removed, gave (June 22) a maximum tension of 8¾ in. Next day about 18 ft. of lateral branches were cut off, leaving a bare main axis about 3½ ft. long, which gave a maximum tension of 5¼ in.: the two experiments were of about equal duration (23 hours).

In connexion with these observations it is worthy of note that in the earlier experiments on the effect of the removal of leaves, I was careful to close the cut surfaces of the branches by means of melted paraffin in order to exclude air; but in the later experiments I omitted this precaution (e. g. Beech shoot *C*) without any apparent effect upon the tension subsequently developed. This was also the case in an experiment with *Helianthus*: a shoot had developed and maintained for some hours a tension of 7½ inches; I cut off the two largest leaves at 3.30 p.m., leaving the wounds open; at 3.45 the tension was still 7½ inches. I then cut off all the remaining leaves, readjusted the apparatus to zero, and obtained the

following record from the bare herbaceous stem, about 2 ft. long:—

June 17,	3.50 p.m.	Indicator =	0 in.	June 18,	9 a.m.	Indicator =	$7\frac{3}{4}$ in.
	4 ,,	,, =	$\frac{1}{2}$,,		10 ,,	,, =	$7\frac{3}{4}$,,
	5 ,,	,, =	$1\frac{1}{2}$,,		11 ,,	,, =	7 ,,
	6 ,,	,, =	2 ,,		Noon	,, =	7 ,,
					2 p.m.	,, =	7 ,,

whence it appears that, in the Beech and the Sunflower at any rate, air cannot penetrate centripetally into the vascular tissues of the plant by means of open wounds at the surface. This I found to be true also of the Yew.

But to return to the question of the relation between leaf-area and suction-force. From the foregoing figures it would appear that the suction-force of a shoot is not immediately dependent upon the leaf-area; and in fact the case of *Helianthus* indicates that an herbaceous stem deprived of its leaves can develope almost as high a suction-force as when the leaves are present. Nor does it appear that the maximum tension is always attained more rapidly with a larger than with a smaller number of leaves. Considering the foregoing data from this point of view we get the following results:—

	No. of leaves.	*Max. tension.*	*Time required.*
Beech. Shoot *A.*	1150	$21\frac{1}{2}$ in.	35 minutes
	820	20 ,,	55 ,,
	550	$18\frac{1}{2}$,,	105 ,,
	355	20 ,,	55 ,,
	245	$18\frac{1}{4}$,,	110 ,,
	leafless stem	$4\frac{3}{4}$,,	160 ,,
Shoot *B.*	320	$13\frac{3}{4}$,,	210 ,,
	239	$9\frac{1}{2}$,,	390 ,,
	43	$5\frac{1}{2}$,,	70 ,,
Shoot *C.*	755	$15\frac{1}{2}$,,	90 ,,
	355	$16\frac{3}{4}$,,	40 ,,
	143	$15\frac{1}{2}$,,	45 ,,
	61	$16\frac{1}{4}$,,	220 ,,
Shoot *D.*	594	23 ,,	270 ,,
	344	$18\frac{1}{2}$,,	370 ,,

Results of a similar kind were afforded by the Yew. A branch (4 ft. long; diam. cut surface = $\frac{1}{2}$ in.) gave (July 3–4)

a maximum tension of 23¾ in.: on the morning of July 4, leafy twigs were removed from it so as to reduce its leaf-area by at least two thirds, and it then gave (July 4–5) a maximum tension of 23 in. On the removal of the whole of its leaves, the tension (July 5–6) reached 18½ in.

However, these figures also show that in certain cases the reduction of the leaf-area did materially lower the maximum tension and prolong the time required to attain it, though not to the extent that might have been expected. The leaf-area is, then, a factor in determining the maximum tension attainable by a shoot, but it is not the only factor; what the other factors may be has yet to be ascertained. It seems to me probable that one other factor is the antecedent condition of the shoot. When the transpiration of a shoot, previously to an experiment, has been greater than its absorption of water, a considerable tension is set up within it; this tension appears to persist as an after-effect, and to enable the shoot to develop a high tension in a subsequent experiment, even though its leaf-area is largely diminished. This is the only way in which I am able to account for some of the observations tabulated on the previous page. The remarkable behaviour of the leafless stem of *Helianthus*, I attribute in part to the fact that the stem is itself an efficient organ of transpiration.

I come now to another enquiry, the enquiry, namely, as to the relation between the life of the shoot and its suction-force. I have not been able to make many observations in this direction, but such as they are, they give some indications.

A branch of Beech (*D*) with 344 leaves gave (June 25) a maximum tension of 18½ in. It was left all night with 10% solution of copper sulphate ($CuSO_4$) being injected into it by a 3 ft. siphon: on the following morning, drops of the copper-solution were exuding from wounds where small branches had been cut off, but the leaves still looked fresh and green: that day a maximum tension of 14¾ in. was attained, and, after being left all night, the tension indicated next morning (June 27) was 14½ in., the fall being due to an

accumulation of gas. It was then dismounted, and placed with its cut end in the copper-solution for forty-eight hours (till June 29). On this date the leaves were discoloured and shrivelled: the shoot was then placed in connexion with the apparatus, and after a prolonged experiment (9.50 a.m.–4.45 p.m.) a maximum tension of 4¼ in. was registered. In a subsequent experiment (July 2–3) with the same branch, after all the leaves had been removed, the maximum tension observed was 4¼ in. Again, a vigorous shoot (*D*) of *Helianthus* gave (July 19–20) a maximum tension of 9¼ in.: it was then injected for twenty-four hours with copper sulphate solution, when it became discoloured and flaccid and gave a maximum tension of 5 in. Hence it appears that the suction-force of a dead shoot is very much less than that of a living one; and more especially that dead leaves contribute but little to the development of suction-force in a shoot, a result which is confirmatory of conclusions recently arrived at by Dixon [1].

When, however, the suction-force of living and dead *leafless* branches is compared, the difference is less considerable; in one case the difference was in favour of the presumably dead branch. The following are illustrative experiments:—

Taxus (Shoot *B*).

a. Bare branch after injection with water (24 hours):—
July 7, 9.30 a.m.–July 8, 9.30 a.m.—Max. tension = 16½ in.

b. Same branch after standing in and injection with $CuSO_4$ solution (80 hours):—
July 11, 7.45 p.m.–July 15, 10 a.m.—Max. tension = 19 in.

Taxus (Shoot *C*).

a. Bare branch after injection with water (16 hours):—
July 28, 9.15 a.m.–3 p.m.—Max. tension = 9 in.

b. Same branch after injection with $CuSO_4$ (16 hours):—
July 29, 9.30 a.m.–4.30 p.m.—Max. tension = 7¾ in.

Helianthus (Shoot *C*).

a. Leafy shoot injected with water (12 hours):—
July 18, 9.30 a.m.–6.30 p.m.—Max. tension = 8¼ in.

b. Same shoot, deprived of leaves, injected with water (14 hours):—
July 19, 11 a.m.–7.30 p.m.—Max. tension = 6¼ in.

[1] Note on the rôle of Osmosis in Transpiration; Proc. Roy. Irish Acad., III, No. 5, 1896, p. 767.

c. Same bare shoot, injected with $CuSO_4$ (14 hours) :—
July 20, 11 a.m.–July 21, 9 a.m.—Max. tension = 4¼ in.

Fagus (Shoot *F*).

a. Bare branch, after injection with water (21 hours) :—
July 24, 9.30 a.m.–6 p.m.—Max. tension = 9¼ in.
b. Same branch, after injection with $CuSO_4$ (13 hours) :—
July 25, 9.15 a.m.–7.30 p.m.—Max. tension = 8 in.

It may be explained that the reason for injecting with water in several of the above observations was to remove any possible after-effect from previous transpiration ; and, further, to render the results of these observations strictly comparable with those obtained with branches injected with solution of copper sulphate.

The question as to the influence of external conditions (heat, light, &c.) on the suction-force of branches is one to which I have been unable as yet to give much attention. I have, however, observed in many experiments that the tension increases more rapidly when a branch is directly exposed to the sun, but I have not specially investigated the point ; I hope to return to it on a future occasion.

In conclusion, I may perhaps venture upon some suggestions as to the bearing of my observations upon the theory of the transpiration-current in entire plants. Whilst it must be admitted that the ultimate motive power is the transpiration of the living or the evaporation from a dead branch, it is by no means clear what the intermediate or subsidiary forms of force may be. Both Askenasy and Dixon attach importance to osmotic processes taking place in the tissues intervening between the conducting wood and the evaporating surface: but it would appear that these processes cannot be of primary importance, since, as my observations prove, a high suction-force can be generated by a branch which is saturated with solution of sulphate of copper. Facts such as this point to the importance of the imbibition-force of the cell-walls—to which Askenasy draws attention—rather than to that of the osmotic force of their contents. Then, again, Dixon and Joly —and Askenasy essentially agrees with them—state that the suction-force conveys water through the wood 'by exerting a simple tensile stress on the liquid in the conduits,' a statement which they cannot be said to have proved, however

plausible it may be. For my own part, I am at a loss to explain on this theory the results which I have obtained as to the suction-force of leafless branches, whether living or dead: for whilst the transpiration of a leafy branch may theoretically suffice to set up a considerable suction-force of the nature of a simple tensile stress on the water in its wood, it is, I think, questionable if the transpiration of a leafless branch would suffice to set up a tensile stress corresponding to the suction-force observed. I am, in fact, inclined to conclude that the imbibition-force of the wood plays a more important part in the conduction of the transpiration-current than is generally admitted at present. It may be that von Sachs' imbibition-theory does not afford a complete explanation of the mechanism of the transpiration-current; but it seems to me that his theory includes some at least of the essential elements of a complete explanation.

But whatever may be the ultimate physical nature of the suction-force, my observations show how considerable is the force which a relatively small shoot can exert; and though there are no data available for a conclusion as to the mode of summation of the suction-forces of the various branches of a tree, yet it is intelligible how that they together constitute a force which suffices to raise water from the roots to the topmost twig. At the same time, I do not consider it probable that the suction-force of branches such as I used, is ever so great in nature as I have found it to be. For in these experiments the branches continued to transpire for hours without absorbing any water; whereas, in nature, the demand of the branches for water would doubtless be supplied by the roots long before the suction-force had attained anything like such dimensions. These experiments illustrate an extreme case.

I am conscious that these observations are by no means complete—in fact they are merely preliminary. I will therefore not at present attempt any further discussion, still less any more definite conclusion.

The Formation of the Sexual Nuclei in Lilium Martagon.

I. Oögenesis.

BY

ETHEL SARGANT.

With Plates XXII and XXIII.

SOME recent zoölogical observations show that in certain cases a transverse division of the chromosomes occurs during one of the karyokinetic divisions immediately preceding the formation of either sexual nucleus[1]. This is sufficiently remarkable, as the solitary exception to the rule of longitudinal fission of the chromosomes during karyokinesis. But it has further been shown that if future research should establish the general law that the formation of a sexual nucleus is always preceded by a transverse division of

[1] See the references (p. 97) in Dr. Haecker's paper: 'The Reduction of the Chromosomes in the Sexual Cells as described by Botanists'—Ann. of Bot., ix. 1895.

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chromosomes, great light will be thrown on the relation of the process of fertilization to the phenomena of heredity[1]. Dr. Valentin Haecker has lately called the attention of botanists to this important subject, and has suggested that they should re-examine the critical divisions in the test case of *Lilium Martagon*[2]. A complete account of the formation of the sexual nuclei in this plant was given in 1891 by M. Guignard in his well-known paper, 'Nouvelles Études sur la Fécondation.' The four spermatogenetic and three oögenetic divisions which follow the reduction in number of the chromosomes are minutely figured and described in this memoir. It is shown in each case that the chromosomes are divided longitudinally. Dr. Haecker has however pointed out that the first karyokinesis on either side is figured as differing somewhat from those which succeed it, and he has hinted that the cause of this distinction may be that during this nuclear division the apparently longitudinal fission of the chromosomes is in fact a transverse one. In view of the theoretical importance of such a discovery, it seemed worth while to make a detailed study of the whole series of nuclear divisions from this point of view, paying particular attention to the long period of growth and development which precedes the appearance of the reduced number of chromosomes on either side. I was encouraged to undertake this work by Dr. D. H. Scott of Kew, to whose advice and sympathy I have owed much throughout its progress. It has been entirely carried out in my laboratory at Reigate.

Before proceeding further it will be necessary to define more exactly the limits of the investigation. The genealogy of the male and female generative nuclei of any given plant of *Lilium Martagon* extends back through a long line of nuclear divisions to the first division of the fertilized ovum from which

[1] For a discussion of Dr. Haecker's hypothesis and its relation to Professor Weismann's Reduction Theory, see Rückert, Die Chromatinreduktion bei der Reifung der Sexualzellen—Ergebn. d. Anat. u. Entw. (Merkel u. Bonnet), 1894.

[2] Vid. Haecker, l. c. pp. 99–101.

the plant arose. It is impossible however to distinguish those nuclear divisions which are in the direct line of descent —the 'germ track'—from those which are not, until we are within a few generations of the sexual nuclei. In the young anther as soon as the archesporial tissue is differentiated we can say with certainty that all the nuclei within it will by repeated divisions give rise to pollen mother-cell nuclei, and therefore four generations later to the male nucleus of the pollen-tube. The ancestry of the ovum cannot be traced back so far. The embryo-sac is a hypodermal cell, distinguished at first from those surrounding it by its median position only. Three successive nuclear divisions within the embryo-sac divide its primary nucleus from the ovum, and only during these three generations can the line of descent be securely followed.

Fortunately it is not necessary for our present purpose that the divisions which will form the ovum should be identified through more than three generations, nor those which are to form the male generative nucleus through more than four. For it is well known that the three karyokinetic divisions which immediately precede the formation of the ovum, and the four preceding that of the male generative nucleus, differ from their predecessors in possessing twelve chromosomes in place of twenty-four. These twelve chromosomes appear in the primary embryo-sac nucleus and in that of the pollen mother-cell respectively just before the formation of the spindle. According to Dr. Haecker's hypothesis, each of the twelve represents two of the chromosomes from the previous division joined end-to-end. The reduction in number implies no corresponding reduction in mass, for the twelve new chromosomes are in fact twelve double segments, equivalent to the twenty-four single segments of the previous karyokinesis. If the subsequent karyokineses effect a longitudinal fission of each chromatic segment as botanists have hitherto asserted, then each segment which goes to build up the nucleus of the ovum or the male generative nucleus is a descendant of one of these double chromosomes and of similar

structure, and all the twenty-four chromosomes of the latest archesporial division are represented in the sexual nucleus. But Dr. Haecker predicts on the faith of zoölogical analogies that one of the three oögenetic nuclear divisions with twelve chromatic segments and one of the four similar spermatogenetic divisions will be found to result in the division of each segment not longitudinally but transversely, and in such a way that each half represents one of the original twenty-four chromosomes. If this should prove true, the descendants of the original twenty-four chromosomes would be divided during this karyokinesis between the daughter nuclei, twelve going to one and twelve to the other. This would be a true reduction division in Professor Weismann's sense. The sexual nucleus thus formed would be constructed of the descendants of twelve only from among the original twenty-four chromosomes.

It is clear that Dr. Haecker's hypothesis rests on three assumptions.

1. That the reduction in number of the chromosomes which has just been described is caused by the joining of twenty-four chromosomes from the previous nuclear division end-to-end in twelve pairs.

2. That in a subsequent nuclear division, before the formation of the ovum on the one hand or of the male generative nucleus on the other, the twelve double segments thus formed are divided not longitudinally but transversely.

3. That this transverse fission corresponds to the junction formerly accomplished between the members of each pair.

Assumptions 1 and 3 are not in themselves improbable, but in order to verify them it would be necessary to identify each chromosome from one karyokinesis to another throughout the intervening resting-stage. This has not hitherto been found possible. The second assumption can be proved or disproved by observation. For in so favourable a case as the anther or embryo-sac of *Lilium Martagon* it is very

possible to follow three or four nuclear divisions in such detail as to place their true nature beyond a doubt. In the following pages I have tried to do this for the three critical divisions of the oögenesis: later on I hope to publish a corresponding set of observations on the four spermatogenetic divisions.

Biological.

The whole process of the formation of the sexual nuclei in *Lilium Martagon* occupies five or six weeks in May and June. At the beginning of this period, transverse sections through a number of buds from a single stalk show that the embryo-sac is differentiated in each of the median ovules of the ovary[1] about the same time that the pollen mother-cells are formed in the anthers of the same flower. The process of oögenesis is finished when the ovum is formed in the embryo-sac, and this takes place as the flower opens. Perhaps a day later the male generative nucleus is formed in the tube of a pollen-grain from the same flower, so that the two processes begin and end together. Nuclear divisions in the pollen mother-cell and embryo-sac of the same flower do not seem to occur at the same time. On the contrary, an alternation of activity is to be observed (vid. Table I).

The first karyokinesis on either side is preceded by a period of growth and development which may be described under two heads.

1. Nucleus in resting condition: it increases in size without material alteration in structure.

2. Process of formation of spirem thread within nucleus, including the stage called synapsis.

[1] The upper and lower ovules in each ovary are always smaller than those in the middle, and usually in an earlier stage of development. The stage of the median ovules is throughout considered typical of that of the ovary.

TABLE I.

Buds.	Spermatogenesis.	Oögenesis.
I. Buds sessile.	Archesporial divisions.	Nucellar divisions.
	Pollen mother-cells differentiated, resting nucleus.	Embryo sac differentiated, resting nucleus.
	Formation of spirem thread in pollen mother-cells.	
II. Pedicels begin to grow. Buds 10–11 mm. long. Anthers green and sessile.	First and second nuclear divisions in pollen mother-cell (1, 2).	
		Formation of spirem thread begins.
III. Filaments begin to grow. Anthers yellow to orange.	Vegetative division in pollen grain (3).	
		Spirem formed.
IV. Buds about 20 mm. long. Anthers deep orange.		First and second nuclear divisions in embryo sac (1, 2).
V. Buds 20–30 mm. long.		Four equal nuclei in embryo-sac.
		Nuclei differentiated in pairs.
VI. Flower opens. Anthers dehisce.	Division of generative nucleus in pollen-tube (4).	Third nuclear division in embryo-sac (3).

METHODS.

The methods of the histologist are necessarily indirect. He is seldom able to observe the structures he describes while they are alive. His only resource is to arrest their movement by killing them as instantaneously as possible, and from a series of the figures thus obtained to attempt a reconstruction of the process of growth. It is therefore essential to bear in mind the possibility of errors arising (1) from deformation produced by the processes of preparation, and (2) from faulty seriation.

Owing to the mass of material dealt with, I have found it impracticable to use consistently more than two fixing methods. My preparations however are in two parallel series

throughout: one set fixed in one of the osmic acid mixtures, sometimes made up with a certain proportion of alcohol in order to ensure penetration, the other fixed in absolute alcohol. The first are stained by Flemming's orange method; the second with a mixture of methyl-green and acid fuchsin or with Renaut's haematoxylic eosin preceded by orseillin. In a few cases other stains have been used for comparison, as Meyer's haemalum. I have compared the two series at every stage and have formed my conclusions on the evidence of both. Further details of these methods are given in an appendix at the end. Moreover, I have been careful throughout to check the conclusions derived from a study of microtome sections by the examination of hand sections from absolute alcohol material. Deformations due to the embedding processes are absent from hand sections cut in pith.

In one case only have I found it possible to examine living tissue with advantage. The contraction of chromatin to one side of the nuclear cavity during the process of formation of the spirem thread, which has been happily termed synapsis by Mr. J. E. S. Moore[1], is treated by some histologists as a natural phenomenon, by others as artefact. As the contraction is invariably seen at the same stage in the development of both pollen mother-cell and embryo-sac nuclei, however prepared, I have for some time considered it as a condition existing during life, though doubtless subject to deformation during fixing and cutting processes. This summer however I obtained sections from fresh anthers on several occasions in which the chromatin of the pollen mother-cell nucleus could be clearly seen collected into a ball on one side of the nuclear cavity. The chromatin was identified by subsequent fixing and staining of the section.

Division of the Vegetative Nucleus.

The nuclei with which we are concerned in this paper are, as already stated, peculiar in exhibiting twelve chromatic

[1] J. E. S. Moore, Science Progress, vol. iii. p. 333.

segments during karyokinesis in place of the usual twenty-four chromosomes. It is essential that any other deviations from the normal type of karyokinesis should be carefully observed and recorded, that they may be fully accounted for and the possibility of their masking a transverse division considered. For this purpose comparison with the details of nuclear division in the vegetative tissues of *Lilium Martagon* is needed. Figs. 1 to 8 represent nuclei belonging to the integuments of the ovule. In the sections from which they are taken the embryo-sac has nearly attained its full size: there can be no doubt therefore that these nuclei are off the 'germ track.' Owing to the flattened shape of the cells, the karyokinetic figures are apt to be spread out in a manner convenient for counting the chromosomes (vid. Figs. 3 and 4). They are also of comparatively large size. I have compared other nuclei from the tissues of anthers and ovaries with these and find that the details of division are identical. When the cell is cylindrical the spindle is not flattened, and then resembles Fig. 9, but the separation of the chromatic segments takes place in just the same way.

The structure of the resting nucleus is most clearly made out in tissue fixed with Flemming's solution and stained by his orange method (Fig. 1). Within the nuclear membrane a network of fine dotted threads is partially masked by cloudy masses of granular substance. In alcohol preparations stained with methyl green and acid fuchsin this substance is bright green. The threads indeed in such a preparation are not visible at all, but the cloudy green substance is seen to be disposed in a coarse mesh-work. Returning to the Flemming preparations, it is a question whether the threads really anastomose with each other. They appear to do so, and there is always a comparatively large dark dot at the junction of two or more threads, but it is possible that this is an optical effect and that the threads only pass very closely behind each other. Thus the possibility of the whole network being in fact composed of one continuous much-tangled filament is not excluded. The whole nuclear cavity is

traversed by these threads, but the patches of cloudy substance, which I shall call amorphous chromatin, seem more numerous towards the periphery. In the larger meshes of the network are several spherical nucleoli of various sizes.

The first stage which can be identified as indicating approaching division is the slender spirem (Fig. 2). The nuclei are decidedly larger than in the resting stage and their structure is very different. The nuclear membrane is still distinct, and within it is a single slender ribbon much convoluted and enclosing within its curves several small nucleoli. No anastomoses are seen, and the ribbon takes a uniform green colour in methyl green. The amorphous chromatin has entirely disappeared. No doubt it has served to feed the ribbon, but I have not been able to find a series of intermediate forms which would link the structure of the resting nucleus to that of the slender spirem. Transitional stages between slender spirem and early spindle (Fig. 4) are easily found. The ribbon becomes shorter and broader so that the convolutions are more simple. At last the nuclear membrane vanishes and the nucleoli disappear. The ribbon then falls into segments. In the stages immediately preceding this the ribbon shows signs of a certain method in its twistings. I have never been able to follow it throughout its course on account of the small size of the nucleus and the number of coils within it. But the general impression left after examining many figures is that there is a definite arrangement of coils resembling that figured by Professor Flemming for the spermocytes of Salamandra [1].

Figures such as that shown in Fig. 4 are extremely common, and in the absence of any direct evidence concerning seriation I was at first inclined to consider them as late stages in which the segments pointing upwards were about to proceed to one pole, and those pointing downwards to the other. On being counted, however, the chromatic segments turned out to be about twenty-four in number, whereas

[1] W. Flemming, Neue Beiträge zur Kenntniss der Zelle, Pl. xxiii. fig. 2.

if the fission had already taken place they should have been about forty-eight [1]. The discovery of earlier intermediate forms, such as that drawn in Fig. 3, and of rather later figures in which the chromosomes though still directed towards either pole show clear longitudinal fission (Fig. 5), confirmed the conclusion that the stage in question (Fig. 4) occurs before the formation of a typical nuclear plate. The ribbon-like character of the segments in Figs. 3 and 4 is marked.

The chromosomes when arranged to form the nuclear plate (Fig. 6) lie in a plane perpendicular to the axis of the spindle. The separation of their segments has begun. Bundles of spindle fibres are attached to each segment near one end, and the segments separate exactly as if they were pulled apart by ropes fixed at this point (Figs. 6 and 7). Each segment as it nears the pole is seen to be hook-shaped (Fig. 7). In Fig. 8 the daughter nuclei are seen to be fringed by the longer legs of the hooks not yet drawn up into them.

The daughter nuclei at first consist of naked coils of chromatic ribbon. By degrees the coils open, a nuclear membrane is formed, nucleoli appear, and the chromatic ribbon lengthens. The nuclei ultimately pass into the resting-stage by a series of changes which I am unable to decipher. The cell-plate and cell-wall are formed in the usual way.

Oögenesis.

While the nucellus is still a mere hump on the placenta, its tissues contain numerous dividing nuclei. The nucleus of any hypodermal cell occupying a fairly median position may be the ancestor of a future embryo-sac nucleus. All these divisions however conform strictly to the vegetative type. The spindle figures differ somewhat in shape, in some cases being equally developed all round the axis of the spindle (Fig. 9), in others flattened as is usual among integument nuclei (Fig. 4). In one such case I was able to count

[1] It is extremely difficult to count the chromosomes is a small spindle figure with perfect accuracy. I have uniformly found about twenty-four chromosomes in nuclei from vegetative tissues of *Lilium Martagon*.

the chromosomes accurately and found twenty-four of them. In all others difficulties of observation prevented me from determining the number with absolute certainty. The question always lay between twenty-three and twenty-four, or between twenty-four and twenty-five.

A median hypodermal cell becomes larger than its fellows some time before the appearance of either integument. The nucleus of this larger cell usually remains for some time in the resting-stage and becomes the embryo-sac nucleus without further change, but not infrequently it divides once again. This accounts for the occasional appearance of twin embryo-sacs which is not infrequent in very young stages. In other cases two nuclei are found in the same embryo-sac long before the regular period for division, and I have seen several instances of twin spindles. One preparation shows a double embryo-sac with four spindles in it which is evidently a case of a twin second division. These anomalous cases are not numerous enough however to account for all the instances in which I have found a nuclear division taking place within an enlarged hypodermal cell. Probably the rule is that one of the daughter-cells increases in size at the expense of the other and becomes the embryo-sac. The karyokinesis itself is of the usual nucellar type.

The structure of the resting nucleus in the very young embryo-sac is precisely similar to that of the nucellar nuclei surrounding it. A network of dotted threads is partially concealed by the substance I have called amorphous chromatin. There is either a single large nucleolus, or more commonly several of different sizes (Figs. 10 and 10 *a*). A glance at Table I will show that the nucleus remains in the resting state until the pollen mother-cells have completed their double division. This probably takes about a fortnight. During this time the nucleus increases in size, almost doubling its diameter, without materially altering in structure (Fig. 11). The threads become thicker and their dots larger, but the latter still form a single row (Fig. 11 *a*). Anastomoses seem to take place, but may be only apparent. The amorphous

chromatin is very much less conspicuous: instead of a heavy deposit of cloudy substance which in methyl-green preparations is of a bright colour and completely masks the thread over which it lies, we have a scanty grey-green precipitate which makes the thread look ragged. This disappearance of amorphous chromatin is partly accounted for by the increase in size of the nucleus, for the area of each nuclear section is nearly four times as great as it was, but there can be little doubt that there has also been a real decrease in quantity of this substance.

If we refer back to the series of preparations bridging this interval, we find that as the nucleus increases in size the network of dotted threads begins to occupy a peripheral position and the amorphous chromatin loses its cloudy look and is heaped more closely over the threads, the meshes being left clear. It is difficult to avoid the suspicion that the chromatin, which was at first in the cloudy probably dilute state which I have described, is now being deposited on the dots in the network.

The nucleoli of the later resting-stage are still spherical and of well-defined outline, but they often look swollen. Careful examination of the chromatic network at this stage reveals drops adhering to it which are rounder and more regular in outline than the fragments of amorphous chromatin. In methyl-green and fuchsin preparations the larger of these dots are distinctly red, and they must therefore be considered nucleolar. These are the first indications of the solution of the nucleolus which follows during synapsis.

The transition between the late resting-stage just described and the contracted state known as synapsis is very instructive, and is marked by (1) the increase in thickness of the chromatic thread and the appearance in it of a double row of dots; (2) the gradual solution of the nucleolus; and (3) the partial disappearance of the nuclear membrane. In the earlier transition stages the nucleolus is still more or less spherical and clearly outlined, but the number of small drops of nucleolar matter (*n*, Fig. 12 *a*) attached to the thread

has very much increased. Perhaps they may be derived from the breaking down of the smaller nucleoli. The thread itself is much thickened, often irregularly: in many places the drops of nucleolar matter adhering to it have elongated in the direction of the thread. This suggests at once that the nucleolar matter is applied to the thread with the purpose of thickening it. Where the thread is of fairly uniform thickness for some distance, two rows of dots can sometimes be made out in it with very high powers. The picture is usually confused by the twisting on itself of the thickened and rather flat thread (Fig. 12 *a*). Where the double row can be made out with any precision, which at this stage is only in preparations from Flemming material stained by the orange method, the ribbon itself is seen to be of a reddish tinge and the dark dots to border it on either side. The nucleolus usually becomes vacuolated before losing its clear outline.

Before finally passing into the contracted state the dotted ribbon just described begins to close round the nucleolus. This has become washy and vacuolated. Large fragments are broken from it: some of these, or the main nucleolus itself, are often sickle-shaped. Smaller fragments are attached to the chromatic ribbon. The outline of the nuclear membrane can hardly be traced.

The nucleus now enters the period of synapsis, in which it remains for some time, probably several days. The whole chromatic thread contracts into a ball, leaving a large part of the nuclear cavity empty. This cavity is somewhat swollen: the membrane can be followed in places, but is often broken and always obscure (Fig. 13). The chromatic ball is always close to one side of the nuclear cavity, and partly surrounds the half-dissolved nucleolus, which is often sickle-shaped (Fig. 13). A general view of the whole structure is best gained from a thick alcohol section stained with methyl-green and fuchsin, as the nucleolar matter is then sharply distinguished from the chromatin. Microtome sections are unsatisfactory, as the chromatic thread as well

as the nucleolus is often displaced and broken by the knife. Tangential microtome sections however give details of the structure of the chromatic thread very well in places.

Two periods of synapsis may be distinguished. During the first the chromatic ribbon grows greatly in width but the dots continue small. (Cf. Fig. 13 *a* with Fig. 13 *b*: 13 *a* is drawn from a nucleus which has just entered the first period of synapsis, and 13 *b* from the nucleus figured at 13 which is near the end of the same period.) This early period is marked by a large and washy nucleolus of irregular outline and by the ill-defined nuclear membrane. In the second period the nucleolus is well defined and spherical, and the nuclear membrane reappears. The ribbon does not become wider, but the dots which border it increase in size. The coils of ribbon also become looser and occupy a larger part of the nuclear cavity. Insensibly the structure of the nucleus becomes that of the well-known spirem stage (Fig. 14. Cf. also Guignard's Figures 44–47, l.c.).

We have now traced the formation of the spirem thread in great detail. The linin ribbon bordered on either margin by a row of chromatin granules can be followed back to the beginning of synapsis. There can be little doubt that this double row is formed just before contraction of the chromatic thread by the fission of the pre-existing single row (cf. Figs. 11 and 12). The linin ribbon at this period and during early synapsis seems to be fed from the partially dissolved nucleolus. I am inclined to think that the solution of the nucleolus is brought about by the entrance into the nuclear cavity of some liquid from the cytoplasm, otherwise it is difficult to explain the temporary disappearance of the nuclear membrane. The contraction of the chromatic coils round the nucleolus is possibly in order that the interstices may be too small to admit of the escape of semi-fluid nucleolar matter. We shall meet with some of these phenomena again at a later stage.

The structure of the nucleus in the spirem stage is too well known to need a long description. In hand sections

from alcohol material I have never found free ends of chromatic ribbon except where the nucleus is touched by the knife[1]. Nor are any anastomoses visible. The ribbon seems to consist of a single coiled and twisted erythrophilous filament, bordered on either margin by a row of cyanophilous dots. Microtome sections about 10 μ thick from Flemming material show structural details of the ribbon very clearly, and it is possible, by careful comparison with the much thicker hand sections, to make sure that we are dealing with a true spirem stage.

We are now approaching the point in the development of the embryo-sac nucleus at which the reduced number of chromatic segments first appears. The history of their development from the single ribbon of the spirem stage is undoubtedly the most important passage in the whole investigation. In order to appreciate the evidence it is necessary to anticipate a little and examine the structure of the mature chromosome[2]. Fig. 20 represents several chromosomes lying loose in the nuclear cavity before the appearance of a spindle. Each is composed of two segments which are twisted round each other. In some cases it is clear that these segments are quite distinct; in others they seem to be joined at one end. It is impossible however to be certain that this appearance is not due to the close approximation of two free ends. At an earlier stage (Fig. 19) the chromosomes are longer, and each segment is an erythrophilous ribbon bordered on either margin by a row of cyanophilous dots. The figure is drawn from a median section in which the chromosomes are clustered round the nucleolus in a very characteristic way (Fig. 19). The ribbon-

[1] The preparation from which Fig. 14 was drawn for instance is not quite perfect. Four free ends are seen in the lower left-hand region, but these all lie in the same plane at the upper surface of the nucleus, and are therefore obviously due to the removal of a tangential section from that surface.

[2] As a matter of convenience I shall speak of the twelve chromatic segments which take part in the first karyokinesis of the embryo-sac nucleus as chromosomes. In doing so I do not mean to express any opinion as to their relation to the twenty-four chromosomes of the previous division.

like character of each segment and the twist of the two segments of each chromosome round each other are even clearer in tangential sections of nuclei at this stage. Such sections often show the structure of one or two chromosomes with diagrammatic precision (Fig. 19 *a*).

The question to be resolved by the examination of stages intermediate between that just described (Fig. 19) and the typical spirem (Fig. 14) is of fundamental importance. The structure of each ribbon-like segment in the immature chromosome precisely resembles that of the spirem ribbon itself. Suppose the spirem ribbon to have fallen into twelve lengths, each of which then doubled on itself, the free ends twisting round each other. The transition to such forms of chromosome as those represented in Figs. 19 and 20 would be accomplished by simple contraction of the twisted lengths of ribbon, together with a fission at the sharp bend in some cases. The fate of the twisted segments can be followed with perfect certainty (Figs. 20–24). During karyokinesis each pair is separated, untwisting during the process, and thus half the segments go to one pole, half to the other. If the two segments of each chromosome had been originally one long length of spirem ribbon, the karyokinesis separating those segments would in fact divide each chromosome transversely.

The process of formation of twelve chromosomes from the continuous spirem ribbon can be followed only in sections thick enough to include the whole nucleus. I have therefore worked out the earlier stages entirely by means of hand sections from alcohol material. Figs. 14–18 are drawn from such preparations, and we are at once struck by the fact that the spirem ribbon begins to split longitudinally while it is still continuous (Fig. 15). It is not separated into lengths until this longitudinal fission is complete, and each length therefore consists of two distinct filaments twisted round each other and not of a single comparatively broad ribbon (Fig. 16). Each filament is necessarily formed of a row of chromatin granules connected by a linin thread.

The double filaments begin at once to contract, and while

doing so they cluster together at one side of the nuclear cavity round the half-dissolved nucleolus (Figs. 17, 18). The nuclear membrane at the same time becomes indistinct. All the phenomena of synapsis have in fact reappeared. After contraction has proceeded for some time each filament shows a double, not a single row of dots. The stage at which this can be clearly made out depends a good deal upon the preparation. That from which Fig. 18 is drawn, for instance, suggests a double row on careful examination, but the thickness of the section prevents absolute certainty. At a rather later stage the double row of dots can be quite clearly seen in microtome sections. I am inclined to believe that the actual fission of the original single row of granules in each filament takes place very shortly after the longitudinal splitting of the spirem ribbon. This is the second occasion on which we have found the phenomena of synapsis associated with the fission of a row of chromatin granules.

The double filaments may now be called chromosomes. They continue to shorten and broaden, and soon acquire the appearance represented in Fig. 19 *a*. Contraction indeed continues up to maturity (cf. Fig. 20 with Fig. 19), but shortly before that time a curious change in the structure of the chromosome takes place. Chromatic dots can no longer be distinguished on segments of linin ribbon. The whole chromosome stains uniformly like chromatin. I cannot even make out whether a fission in each mature segment corresponds to the interval between the two rows of chromatin granules. Each segment looks perfectly homogeneous.

One effect of this change is to make it much more difficult to distinguish between the segments of a single chromosome. The twist of the segments on each other naturally becomes closer during contraction, but while the outline of each was traced by a row of dark dots the compound character of the chromosome was clear. After the segments have become uniform in colour it is only in favourable cases that their twist on each other can be made out at all (Figs. 20, 21).

Little remains to be said as to the behaviour of the

chromosomes during karyokinesis. Traces of their grouping round the nucleolus persist until the nucleolus and nuclear membrane disappear together (Fig. 20). The spindle appears at first as an irregular sheaf of fibres which quickly becomes symmetrical. Figs. 21 and 22 (*a*—*c*) show how the chromosomes are attached to the bundles of spindle fibres. The point of attachment is sometimes about the middle of the chromosome, sometimes near one end[1]. The segments untwist from each other as they are pulled apart. This probably accounts for their strained and knotted look just before separation (Fig. 23). In the diaster (Fig. 24) the segments are generally V-shaped, their angles directed towards either pole[2].

The large size of the spindles and the reduced number of chromosomes makes it fairly easy to count the latter in thick sections. Ambiguity often arises however from the different ways in which the chromosomes separate. It is sometimes impossible to distinguish between a single chromosome separating in the middle and two adjacent end-splitting ones. In thin serial sections one or more chromosomes are commonly shattered by the knife, and the fragments can seldom be pieced together with certainty. Nevertheless, I was able in fourteen cases to count twelve chromosomes in the nuclear plate. In many other instances from among some hundred spindles the question lay between eleven or twelve chromosomes, and in a few it was doubtful whether there were twelve or thirteen. I have no instance of a number so low as ten or so high as fourteen being even suggested.

The daughter nuclei first appear as two tight knots of chromatic ribbon. Their construction from the diaster segments cannot be followed. Spherical drops of erythrophilous substance commonly appear in the cytoplasm about this

[1] A typical example of a chromosome separating from the middle is shown in Fig. 21. It is instructive to compare it with a similar chromosome applied to the nucleolus in Fig. 20. This suggests that nucleolar matter serves, if not as material for spindle fibres, at least to solder them to chromosomes.

[2] I have one preparation in which the diaster segments are hooked as in the vegetative nucleus.

time. Later on, as the coils of the daughter nuclei open out, the number of drops increases, and some are found inside the nuclei (Fig. 25). There can be little doubt that we here have drops of nucleolar substance from which the new nucleoli are being reconstructed. The coils of chromatic ribbon are at first coloured uniformly like chromatin, but later on they show the familiar structure of a linin ribbon bordered with dark dots (Fig. 25). No doubt the dots are granules of chromatin, but their identity with those bordering each segment of the immature chromosome (Fig. 19 *a*) cannot be traced. The cell-plate is still clear between the nuclei. It finally disappears when they have passed into the resting-stage (Fig. 26).

We may now sum up the conclusions drawn from our examination of the first division of the embryo-sac nucleus. The chromosomes have been identified with lengths of the ribbon so clearly shown in the well-known spirem stage. Each segment of a single chromosome represents one of the two parallel rows of granules found in such a length of ribbon. It has never been doubted that these arose by fission of a single row at an earlier stage[1], and the history of their development confirms this view. The karyokinesis therefore which separates the two segments of each chromosome from each other does in fact divide the chromosomes longitudinally.

The history of the second longitudinal fission—that which takes place within each segment—is not so satisfactorily completed. It is not certain that this fission persists in the mature chromosome (vid. ante, p. 461). Even supposing that it does, and that the chromatin granules mentioned as appearing in the dispirem can be identified with the granules of the immature chromosome, all trace of fission is again lost in the resting-stage of the daughter nuclei. It is not impossible indeed that the slender thread of this stage bears a double row of granules, but there is no evidence that it does so. The existence of four rows of granules in the immature

[1] Guignard, l. c., p. 183.

chromosome however is interesting, since it recalls the structure of the corresponding stage in the spermocyte of *Ascaris megalocephala*. Here also Dr. Brauer has traced the four rows to a double longitudinal fission [1].

The two nuclei which now occupy the embryo-sac (Fig. 26) soon show signs of approaching division. They increase in size, and the ragged-looking network characteristic of the resting state gives place to a neat spirem ribbon. Some preparations show this ribbon clearly bordered on either side by a row of dark dots. It very soon becomes of a uniform colour. At first both nuclei possess a nuclear membrane and nucleoli, and even at this early stage a difference in size between them may often be observed (Fig. 27). The chalazal nucleus is commonly larger than that at the micropylar end. Both nuclei are sometimes surrounded by a comparatively clear space divided from the cytoplasm by a granular boundary (Fig. 27). After disappearance of the nuclear wall this clearer space persists, and well-marked radiations are found in it. A washy nucleolus can still be perceived among the tangled chromatic coils (Fig. 28).

Segmentation of the naked spirem ribbon takes place while it is still much tangled and of irregular breadth (Fig. 29). The spindle-fibres have appeared and seem to stretch the figure in one direction, so that the young chromosome is much pulled out in that part which lies along the spindle-fibres and twisted or knotted at the free ends. By degrees the chromosomes contract to uniform breadth, though their ribbon-like character is still apparent. The spindle has also become better defined and more regular, and each chromosome lies on it with one end in the equatorial plane, the other extended to one of the poles.

The chromosomes can now be counted at least roughly, and the well-known distinction between the nuclei becomes apparent. There are about twelve chromosomes in the micropylar nucleus, but commonly not less than twenty-four in the

[1] Brauer, Zur Kenntniss der Spermatogenese von Ascaris megalocephala (Fig. 25).

chalazal one. I have not been able to trace any difference beyond that of size between the two nuclei during segmentation. The chromosomes when fully formed are quite as large in the chalazal as in the micropylar nucleus. Their number can be determined most easily somewhat later when the nuclear plate is formed. Exact results however are rarely to be obtained on account of difficulties of the same kind as those experienced in the previous division. They are increased in this case by the small size of the spindles and slender shape of the chromosomes. I have found but two micropylar spindles in which there could be no doubt about the number of chromosomes. In each case it was twelve. Twenty other micropylar nuclei allowed of approximate counting. In sixteen there were either eleven or twelve chromosomes, and in two either twelve or thirteen chromosomes. Of the two remaining nuclei one possessed either eleven, twelve, or thirteen, and one either ten, eleven, or twelve chromosomes. The exact number of the chromosomes in the chalazal nucleus is of less importance, and the results much more uncertain on account of the crowded nuclear plate. Among twenty-five spindles in which the chromosomes could be approximately counted, one had about twenty, nine about twenty-four, five about twenty-eight, and ten about thirty-two chromosomes.

Our object being to trace the formation of the ovum, we are strictly concerned with the micropylar nucleus only, and Figs. 29–31 are drawn from it. The chalazal nucleus however goes through the same changes at the same time; indeed, the correspondence between the two nuclei is very striking. They are always in exactly the same stage of division.

The chromosomes gradually move into an equatorial plane to form the nuclear plate, but before this is accomplished the longitudinal fission of each can sometimes be observed. The separation of the segments takes place exactly as in the vegetative nucleus (cf. Figs. 30 and 31 with Figs. 6 and 7). Occasionally however a chromosome begins to open about the middle of its length (x, Fig. 30), just as those of the

previous karyokinesis so frequently do. The diaster segments are commonly hooked, but occasionally a V-shape is found (x, Fig. 31). The reconstruction of the daughter-nuclei goes on in the same way as after the first karyokinesis (cf. Fig. 33 with Fig. 25). The pairs of nuclei may differ greatly in size (Fig. 32) or be nearly equal (Fig. 33). This seems to depend somewhat on the size of the embryo-sac. When it is comparatively long at the time the second karyokinesis takes place, the two dividing nuclei are widely separated, and the micropylar nucleus is commonly much smaller than the chalazal one. This difference is shown most clearly during the later stages of division. By the time the daughter nuclei have reached the resting-stage they are already differentiated in pairs. The two egg-shaped nuclei at the micropylar end are cut off from the larger flattened chalazal nuclei by the formation of vacuoles as the embryo-sac lengthens, and no further change takes place before the third karyokinesis (cf. Fig. 37). In short embryo-sacs the two dividing nuclei are often of equal size (Fig. 33), and the four resting nuclei are similar and placed at nearly equal intervals from each other (Fig. 34). The increase in length of the embryo-sac then takes place later.

In such embryo-sacs as these I have found some curious stages before differentiation of the nuclei in pairs. They are too numerous to be treated as pathological. The four similar nuclei are found in all the early stages of division (Figs. 35, 36), but they never reach the stage of regular spindle formation, though I have twice seen indications of spindle-fibres surrounding the well-differentiated naked spirem. These spirem figures are always arranged in the same way within the embryo-sac—a single nucleus near the micropyle and a group of three at the chalazal end. I have never found embryo-sacs with eight nuclei in the neighbourhood of these ovules, and do not believe that the division thus indicated comes to anything. As serial sections from older ovaries show no abortive or unhealthy ovules, I suppose that the four nuclei after making this false start return to the resting-

stage, and become differentiated in pairs in the usual way (Fig. 37).

Between the end of the second karyokinesis and the beginning of the third, some time elapses (vid. Table I), during which the embryo-sac increases in length. Vacuoles are formed which divide the pairs of nuclei from each other. The two micropylar nuclei continue egg-shaped and small: the lower ones are larger and of less regular shape. Very often the upper chalazal nucleus is flattened as if pressed between the lower one and a vacuole, while the lower chalazal nucleus is of a shape determined by that of the end of the embryo-sac into which it fits (Fig. 37). I have described elsewhere the curious difference apparent between the lower chalazal nucleus and the other three nuclei during the third karyokinesis in the embryo-sac[1]. It is conspicuous as soon as the nuclear division begins. While the spirem ribbon in the three upper nuclei is beautifully differentiated, the lowest nucleus, though swollen, is still in the resting condition (Fig. 38). It then proceeds to divide by the direct or amitotic method (Figs. 39–41). The chromatin aggregates at either end as the nucleus lengthens, and the linin network between the masses of chromatin is drawn out in threads. In the later stages of division these give the figure a strong resemblance to a badly preserved dispirem (Fig. 41). The nucleolus disappears during the process: sometimes a washy mass of semifluid nucleolar matter can be seen outside the nucleus (*n*, Fig. 39).

Meanwhile the karyokinetic division of the three upper nuclei goes on quite typically. We are chiefly concerned with the lower micropylar nucleus which will give rise by its division to the ovum and to the upper polar nucleus. The chromosomes however divide and their segments separate in exactly the same manner in all three figures. The process is also simultaneous, so that if one section of a series shows a single spindle in a particular stage, we are certain to find two others in neighbouring sections at exactly the same point

[1] E. Sargant, on direct Nuclear Division in the Embryo Sac of Lilium Martagon– Ann. of Bot., x. 1896, p. 107.

of development. Segmentation takes place just as in the second karyokinesis. The chromosomes are at first twisted, but become straighter and arrange themselves in the usual way so as to mask the spindle (cf. upper nucleus in Figs. 39, 40). Longitudinal fission of each chromosome appears about the time that the nuclear plate is formed, and separation of the segments follows according to the vegetative type (Figs. 42, 43). Occasionally a chromosome is found in which separation has begun near the middle, but this is not so common as during the corresponding period of the second karyokinesis.

As usual, only a small proportion of the whole number of preparations have spindle figures sufficiently clear and perfect for satisfactory counting of the chromosomes. And as I have not so many preparations of the third nuclear division in the embryo-sac as of the two previous ones, the whole number of countings is not so great. One spindle figure of each micropylar nucleus shows twelve chromosomes quite clearly. In seven other cases there are about twelve chromosomes in the nuclear plate of each. Among twelve comparatively clear chalazal spindles, five have about twenty-four chromosomes, and seven a larger number, not exceeding thirty-four in any case.

Reconstruction of the daughter nuclei proceeds as after previous divisions. Four nuclei are in a group near the micropyle, four at the chalazal end. One of the micropylar group is the nucleus of the ovum. With its formation the study of the oögenetic nuclear divisions ends.

Conclusions.

As regards the main object of this investigation the conclusion is clear. The chromosomes are divided longitudinally during each of the three nuclear divisions which precede the formation of the ovum. The first of these divisions does in fact differ considerably from the other two, which follow the vegetative type of karyokinesis, but this difference cannot be interpreted as concealing a transverse fission of the chromosomes. I am at a loss to explain the meaning of this

deviation from the ordinary type of karyokinesis, but its nature and extent are worth consideration.

The characteristic features of the first division of the embryo-sac nucleus are (1) the long period of growth and development before the formation of the spirem thread and its division into chromosomes, and (2) certain peculiarities of form which characterize the chromosomes while separation of the segments is taking place. To this variation in shape of the chromosomes little importance need perhaps be attached. They are not alike when the spindle first appears. Each is formed of two segments much twisted on each other, but the number of twists, their tightness, and the disposition of the free ends vary in different chromosomes. The spindle fibres commonly attach themselves to one of the loops in the double chromosome, or sometimes to one end. It is clear that the two segments of a chromosome will not separate in the same way when they are pulled apart from the middle as when the loop where separation begins is near one end or the spindle fibres are fixed to the ends themselves. Besides this, it must be considered that the segments of each chromosome untwist from each other during the process of separation, and this of itself gives rise to much variation in shape, and often causes two adjacent chromosomes in the same nuclear plate to look very different (cf. Fig. 22, *a–c*). The segments of a chromosome during karyokinesis of the vegetative type separate regularly from one end and on one uniform plan, probably because they are little if at all twisted on each other (Fig. 5), and do not appear until the chromosomes are in order on the spindle. Thus the peculiarities of the mature chromosomes in the first division of the embryo-sac nucleus can be traced back to early stages in its development.

The history of that development has already been told in detail.

The nucleus passes through four phases — resting-stage, synapsis, spirem, and segmentation. It is interesting to enquire whether parallels can be found to any or all of these stages in the history of the vegetative nucleus.

1. The *resting-stage* is the same in both. The young resting nucleus of the embryo-sac cannot be distinguished from the nucellar nuclei which surround it. Later on it surpasses them in size, but in structure is essentially the same.

2. The condition of *synapsis* is peculiar to the primary embryo-sac nucleus, and to that of the pollen mother-cell. Its characteristic features are contraction of the chromatic thread to one side of the nuclear cavity, partial solution of the nucleolus, and partial disappearance of the nuclear membrane. I have never met with a similar contraction either in vegetative nuclei or in those produced by embryo-sac divisions. Partial solution of the nucleolus may sometimes be observed in the four nuclei of the embryo-sac about the stage of Fig. 34. Possibly this may be an indication of approaching division. It should be observed however that the contraction, which is by far the most striking feature of synapsis, may perhaps be of little importance compared to the other characters. If, for example, it were merely a device to prevent the half-dissolved nucleolar matter from escaping into the cytoplasm, it would be useless in a smaller nucleus, for the meshes of the chromatic thread would be fine enough to serve this purpose without contraction. Partial solution of the nucleolus together with a vaguely outlined membrane are characters which in such a nucleus would readily escape observation.

3. The structure of the *spirem* stage in the embryo-sac nucleus is very different from that of the vegetative spirem. Perhaps they should be distinguished by different names. They both possess a coiled chromatic ribbon and nucleoli within the nuclear membrane. The ribbon of the vegetative spirem however stains uniformly like chromatin, while the spirem ribbon of the primary embryo-sac nucleus is erythrophilous, and bordered on either margin by chromatin granules. I have searched in vain among vegetative nuclei for a slender spirem in which a similar differentiation may be observed. It is very possible however that such a stage may exist while

the spirem ribbon is still so narrow that in such small nuclei as we are now considering the double row of dots cannot be distinguished from a single row. I am the more inclined to believe it, as the double row of dots is found in the spirem stage of the second and third embryo-sac divisions, which in all other respects conform to the vegetative type (ante, p. 464). Supposing that this is the case, the difference between the vegetative spirem and that of the first embryo-sac nucleus would amount to this, that the change from a dotted to a homogeneous ribbon occurs early in the history of the vegetative spirem, while in the primary embryo-sac nucleus it is postponed until after segmentation has taken place.

4. *Segmentation* of the spirem ribbon, that is its division into lengths by transverse fission, occurs in every karyokinesis. In that of the primary embryo-sac nucleus it is preceded by longitudinal fission of the whole spirem ribbon, whereas in the vegetative nucleus no traces of longitudinal fission can be perceived until the chromosomes are in position on the spindle.

On the whole, we may conclude that the real departures from the vegetative type of karyokinesis shown by the primary embryo-sac nucleus occur during the earlier stages of development. They can only be partially referred to our ignorance of those stages in the history of the vegetative nucleus. Such distinctive features occurring at so critical a period as that immediately preceding the appearance of the reduced number of chromosomes cannot be without meaning. They certainly do not usher in a transverse division of chromosomes. It is difficult to believe that so long a preparation is needed in order to accomplish the mere reduction in number. For during the second karyokinesis in the embryo-sac, which follows on the first almost without interval, the chalazal nucleus exhibits from twenty-four to thirty-two chromosomes in its nuclear plate, though formed itself from the twelve chromosomes of the first division. The true explanation is still to be found.

The question of the identity of the chromosomes throughout

a series of karyokinetic divisions has been much debated. My observations do not exclude the possibility of such identity. If the network of the resting nucleus be constructed of a single much convoluted filament, and if this filament possess all the properties characteristic of a chromosome, then the chromosomes may preserve their identity throughout a resting-period. We must consider the substance called amorphous chromatin to be of the nature of a food-stuff. I am more disposed to think that the chromosomes are re-cast during each resting-period, part of their chromatin appearing in the amorphous form for redistribution on the spirem ribbon. With regard to the allied question of the relation between dotted ribbons such as those of the immature chromosome in Fig. 19 *a*, and the homogeneous ribbons of a later stage, there is little to say. The change takes place suddenly, and no differentiation can be observed in the ribbon afterwards. A dark line can indeed be traced along its edge, but this is probably due to double refraction. There is no evidence to indicate that the identity of the dots is preserved, but it is not impossible that an incomplete fission may correspond to the plane which separated the two rows of dots.

In describing the phenomena of synapsis I have traced a connexion between the nucleolus and the linin both of the spirem ribbon and of the immature chromosomes. Drops of nucleolar matter are frequent in the cytoplasm during the early stages of spindle formation, and Mr. J. E. S. Moore once pointed out to me in a preparation from the second embryo-sac karyokinesis that a number of these small drops or granules were applied to each chromosome in the nuclear plate. I have frequently observed the same thing since in other preparations of that stage. It would certainly suggest that the nucleolus plays some part in the growth of the chromosomes. The history of their formation from the first embryo-sac nucleus shows that they must contain a great deal of linin.

Little has hitherto been said of the achromatic structure of

the spindle. The chromosomes behave during karyokinesis as if their segments were drawn apart by the spindle-fibres to which they are attached[1]. The spindles themselves when first formed are irregular in shape, sometimes triangular. My observations throw no light on their origin, and I have never been able to trace centrosomes at their poles.

The curious formation of the lower antipodal nuclei by a process of direct or amitotic division is a pretty illustration of the general tendency among the nuclei of degenerating tissues to divide in that way. Mr. H. H. Dixon has found amitotic divisions in the endosperm of *Fritillaria imperialis*[2]. The vegetative nucleus of the pollen grain goes a step further and does not divide at all.

APPENDIX ON METHODS.

A. *Fixing.*

The ovaries, gathered between 10 A.M. and 2 P.M., were cut, and the cuticle was partly removed at once. They were then suspended in alcoholic Flemming's solution for 1½ or 2 hours. The solution was made up as follows:—

1% chromic acid in water . .	3 c.c.
1% osmic acid	8 c.c.
Glacial acetic acid	2 c.c.
Absolute alcohol	27 c.c.

It will only keep for a few hours in the dark. The ovaries were then removed to ·5% aqueous solution of chromic acid for eighteen to twenty-four hours. This gives them a good

[1] In a communication to the Royal Microscopical Society (1895) I have expressed a different opinion concerning the first division of the pollen mother-cell nucleus. I do not now think that the contorted appearance of the chromosomes implies automatic movement on their part, but rather that it is due to the untwisting of the segments during separation.

[2] H. H. Dixon, Proc. Royal Irish Acad., 3rd ser., vol. iii.

consistency for cutting. They were washed and placed successively in 30%, 50%, 70% alcohol at intervals of twenty-four hours, and finally removed to methylated spirit. Those needed for immediate embedding were left in spirit for a day or two, the others preserved in a mixture of about equal parts of alcohol, water and glycerin. The transition from water to spirit was always made in the dark to prevent precipitation of chromic acid.

Other ovaries were placed for a couple of days in absolute alcohol, then transferred to methylated spirit for a week, and preserved in the glycerin mixture.

B. *Embedding and cutting.*

I have followed Dr. M. Heidenhain's embedding process with bergamot oil as a penetrating agent. It easily goes bad, and then the sections crumble under the knife. Care must be taken to change the oil as soon as it begins to turn yellow and smell rancid. I found paraffin melting at 55° C. sufficiently hard.

C. *Staining.*

1. Flemming's orange method for material fixed in Flemming's solution.

The sections were left about thirty hours in 1% solution of safranin (Grübler's 'spiritus-löslich') in absolute alcohol diluted with its own bulk of water. They were washed out in 50% alcohol, slightly acid, then neutral 30% alcohol and transferred to distilled water. In some cases they were placed for from three to five minutes in 1% solution of potassium permanganate as a mordant, in others transferred at once to ·25% solution of gentian violet in water. After remaining in the gentian violet for two to four hours they were washed out successively in 2% aqueous solution of Grübler's 'orange G,' 1% solution of 'orange G' in 50% alcohol, and methylated spirit; dehydrated, and cleared in clove oil.

2. Renaut's haematoxylic eosin for alcohol material.

The sections were left all night in a solution of two to three drops orseillin extract diluted with 100 c.c. of water.

They were then rinsed and placed in a very dilute solution of Renaut's haematoxylic eosin in ·1% aqueous solution of potash alum. This is useless as soon as it goes acid: great care was taken that it should be in good condition, violet not red by transmitted light. The sections may be left in twenty-four hours if the solution is sufficiently dilute. They were always washed afterwards in several changes of hard tap-water to keep them alkaline, and the dilute alcohols through which they passed were made up with tap-water. The orseillin stain is washed out slowly by dilute alcohol.

EXPLANATION OF FIGURES IN PLATES XXII AND XXIII.

Illustrating Miss E. Sargant's paper on the nuclei of *Lilium Martagon*.

The figures marked × 290 and × 585 were drawn under Zeiss' apochromatic hom. im. objective 2. m.m. focal length, N. A. 1.30 with eye-pieces 2 and 4 respectively. Those marked × 1000 and × 1050 were drawn under Zeiss' apochromatic hom. im. objective 1·5 m.m. focal length, N. A. 1·30, with eye-piece 6. The figures were outlined with the camera lucida, and their magnification calculated in the usual way by projection of the scale of a stage micrometer on to the paper scale supplied by Zeiss. The difference between the magnifications of those marked × 1000 and × 1050 respectively is due to a difference in the height of the drawing-board on to which the outline was projected.

Plate XXII. *Lilium Martagon.*

Figs. 1–8. Vegetative nuclei from the integuments of ovules.

Fig. 1. Resting nucleus. × 1000.

Fig. 2. Nucleus in slender spirem. × 1000.

Fig. 3. Nucleus just after segmentation. In this and following figure the chromosomes are ribbon-like. × 1000.

Fig. 4. Early spindle. × 1000.

Fig. 5. Eight chromosomes showing longitudinal fission from an early spindle. × 1000.

Fig. 6. Three chromosomes from a nuclear plate. Separation is beginning from one end. × 1000.

Fig. 7. Chromosomes from a late nuclear plate. × 1000.

Fig. 8. Late diaster.

Fig. 9. Nucleus from young nucellus.

Fig. 9. Nucleus in early spindle from hypodermal cell occupying a median position in a young nucellus and therefore a possible ancestor for the embryo-sac. ×1000.

Figs. 10–12 *a*. Nuclei from embryo-sac.

Fig. 10. Median optical section of resting primary nucleus. ×585.

Fig. 10 *a*. Tangential view of same. The chromatic thread shows a single row of dots. ×1050.

Fig. 11. Later resting stage. *n* nucleolus. ×585.

Fig. 11 *a*. Part of chromatic thread (x, fig. 11), more highly magnified. It still shows a single row of dots.

Fig. 12. Transition to synapsis. The thread has thickened and the dots are larger. ×585.

Fig. 12 a. Part of chromatic thread more highly magnified. The thickened thread shows a double rows of dots. *n n* lumps of nucleolar matter.

Plate XXIII. *Lilium Martagon.*

Figs. 13–43. Nuclei from embryo-sac.

Fig. 13. Primary nucleus in synapsis. Chromatic thread contracted. Nucleolus sickle-shaped. Membrane obscure. ×585.

Fig. 13 *a*. Part of thread from younger nucleus during synapsis more highly magnified. It is hardly broader than that shown in Fig. 12 *a*.

Fig. 13 *b*. Part of thread from tangential section of nucleus shown in Fig. 13, magnified to same extent as Fig. 13 *a*. It is broader than that of Fig. 13 *a*, but the dots are no larger.

Fig. 14. Primary nucleus in spirem. ×585.

Fig. 15. Similar nucleus rather later. The chromatic ribbon begins to split longitudinally while still continuous. ×585.

Fig. 16. Similar nucleus just after segmentation. ×585.

Fig. 17. Primary nucleus soon after segmentation. The long double segments show a tendency to cluster round the washy nucleolus, and the membrane becomes obscure. ×585.

Fig. 18. Primary nucleus a little later. The long segments have contracted and cluster round half-dissolved nucleolus. Each semi-segment shows signs of longitudinal fission. The membrane is obscure. ×585.

Fig. 19. Primary nucleus at stage a good deal later than that shown in Fig. 18. Median section of series. Each immature chromosome consists of two ribbon-like segments which are bordered on either margin by a row of chromatic granules. Membrane broken, perhaps accidentally. The chromosomes are clustered round the nucleolus, which now has a definite outline. ×585.

Fig. 19 *a*. Single immature chromosome at stage corresponding to that of Fig. 19, but from a tangential section of another nucleus. ×1050.

Fig. 20. Primary nucleus with mature chromosomes just before disappearance of nuclear membrane. Traces of the cluster round the nucleolus persist. ×585.

Fig. 21. First karyokinesis. Five chromosomes from early spindle. Drops of nucleolar matter scattered over spindle and cytoplasm. ×585.

Fig. 22 (*a-c*). Three successive sections of a single nuclear plate (first karyokinesis). There are unmistakably twelve chromosomes. ×585.

Fig. 23. First karyokinesis. Segments of three chromosomes are almost separated. × 585.

Fig. 24. Diaster of first karyokinesis. The upper and lower segments belong to different chromosomes. × 585.

Fig. 25. Dispirem of first karyokinesis. Two rows of dots in chromatic thread. Reconstruction of nucleoli is taking place. × 290.

Fig. 26. Embryo-sac with two resting nuclei. × 290.

Fig. 27. Embryo-sac with two nuclei in early spirem stage. Membrane and nucleoli intact. × 290.

Fig. 28. Embryo-sac with two nuclei in late spirem. Membrane has vanished: traces of nucleoli remain. × 290.

Fig. 29. Second karyokinesis. Micropylar nucleus during segmentation. × 585.

Fig. 30. Second karyokinesis. Micropylar nucleus has formed nuclear plate. Seven chromosomes are shown in this section which cuts the spindle rather obliquely. The chromosome marked *x* is dividing from the middle. × 585.

Fig. 31. Second karyokinesis. Chromosomes from late nuclear plate of micropylar nucleus. × 585.

Fig. 32. Embryo-sac with two nuclei in early dispirem. Chalazal pair larger than micropylar pair. × 290.

Fig. 33. Embryo-sac with two nuclei in late dispirem. Both pairs the same size. × 290.

Fig. 34. Embryo-sac with four resting nuclei of equal size. × 290.

Fig. 35. Embryo-sac with four similar nuclei in early spirem. × 290.

Fig. 36. Embryo-sac with four similar nuclei in late spirem. × 290.

Fig. 37. Embryo-sac with four nuclei differentiated in pairs. × 290.

Fig. 38. Embryo-sac with three upper nuclei in slender spirem. Lowest nucleus resting. × 290.

Fig. 39. Chalazal end of embryo-sac. Upper nucleus has formed early spindle: lower one has begun to divide amitotically. *n* nucleolus. × 290.

Fig. 40. Chalazal end of embryo-sac. Upper nucleus still in early spindle stage but later than that of Fig. 39: lower nucleus constricted. × 290.

Fig. 41. Chalazal end of embryo-sac. Upper nucleus in early dispirem. Lower nucleus in pseudo-dispirem. × 290.

Fig. 42. Third karyokinesis. Six chromosomes from nuclear plate of micropylar nucleus. × 585.

Fig. 43. Third karyokinesis. Three chromosomes from late nuclear plate of lower micropylar nucleus. × 585.

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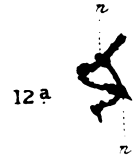

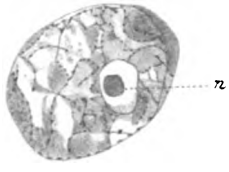

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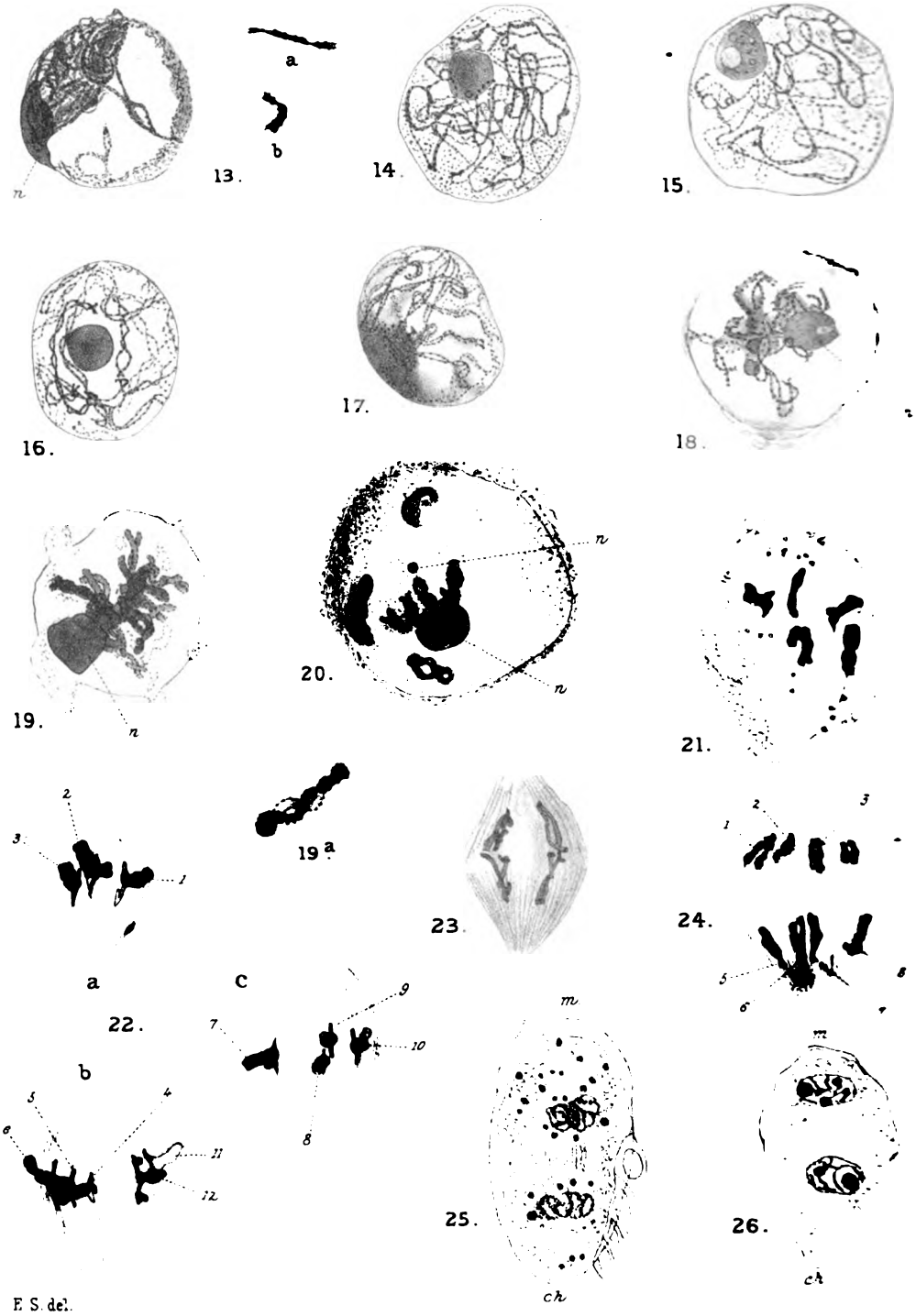

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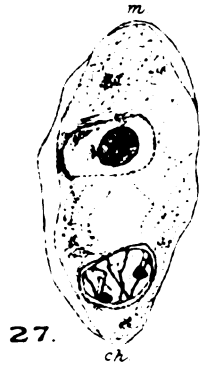

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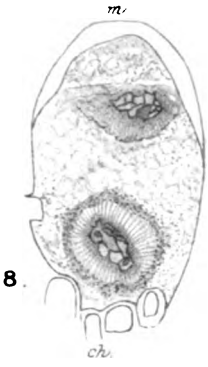

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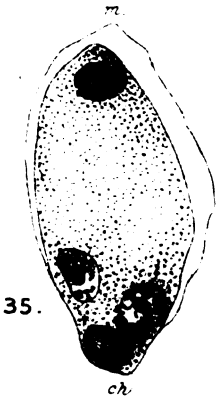

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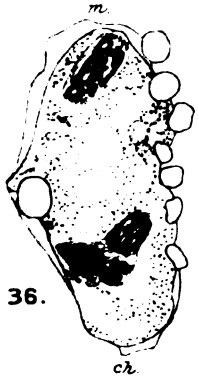

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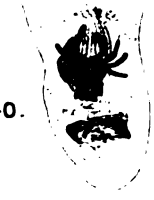
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NOTES.

ON FERTILISATION, AND THE SEGMENTATION OF THE SPORE IN FUCUS[1]. By J. Bretland Farmer, M.A., Professor of Botany at the Royal College of Science, and J. Ll. Williams, Marshall Scholar at the Royal College of Science, London.—The object of the present communication is to give an account of the chief results of an investigation into the processes connected with the formation and fertilisation of the oospheres and the germination of the spore in *Ascophyllum nodosum*, *Fucus vesiculosus*, and *Fucus platycarpus*. The more obvious details of development have been especially studied by Thuret, and later by Oltmanns. But neither of these writers paid any special attention to the behaviour of the cell-nuclei, nor did they succeed in observing the actual process of fertilisation. Behrens has communicated an account (Ber. d. Deutschen Bot. Gesel., Bd. IV) of some researches made by himself on the fertilisation of the oospheres, but we are unable to accept his conclusions for reasons shortly to be recounted.

The material for these investigations was obtained in London from Bangor, Plymouth, and Jersey, but it was compared with other material collected, and fixed at the seaside at Bangor, Weymouth, and Criccieth. Furthermore, all the growing apices and conceptacles for sectioning were collected by one of us directly at the three last-named places. Some samples were gathered between the tides, and fixed at once, others were first kept for a time in salt water; the best results, however, were obtained from plants collected in a boat about two or three hours after the tide had reached the plant, and also from other plants taken a short time before they were left exposed by the ebb tide.

In order to study the stages of fertilisation and germination, male

[1] Paper read before the Royal Society, June 18, 1896.

and female plants were kept in separate dishes, and were covered over so as to prevent drying up. This method gave far better results than those more usually advocated. On the appearance of the extruded sexual products, the female receptacles were placed in sea-water, and after the complete liberation of the oospheres, a few male branches with ripe antherozoids were first placed in a capsule of sea-water until it became turbid owing to their number. If on examination the antherozoids proved to be active, small quantities were added to the vessels containing the oospheres. The latter were then fixed at intervals of five minutes during the first hour, and then at intervals of fifteen minutes, up to six hours after the addition of the antherozoids. After that, samples were killed at longer intervals up to three days, and this was continued till we had material fixed at all stages for the first fortnight. At first we used sea-water in which to keep the embryos growing, but a proper solution of Tidman's sea-salt was found to answer quite as well.

For fixing, we tried the following reagents—chrome-alum, picric-alum, Mann's picro-corrosive, corrosive sublimate, and acetic acid; these were all dissolved in sea-water; absolute alcohol, Flemming's and Hermann's solutions, and the vapour of osmic and formic acids. The Flemming's (strong formula) and Hermann's solutions were diluted with equal parts of sea-water. The first three fixatives were unsuccessful, acetic-corrosive yielded fair nuclear figures, but the material proved very brittle, and the spores were somewhat distorted. A portion of the cytoplasm was disorganised and the polar radiations were not preserved. Absolute alcohol fixed the oospheres and newly fertilised spores without distortion, but was useless for all other stages. Vapour fixing with osmic acid succeeded better than any of the preceding reagents, but was greatly inferior to either Hermann's or Flemming's solutions in preserving the protoplasmic structure in an unaltered state.

After the material had been fixed it was dehydrated and passed in the usual way into paraffin, the temperature of which was not allowed to exceed 50°C., and it was then cut with the microtome. The sections were stained with Heidenhain's iron-hæmatoxylin, with Flemming's triple stain, and a large number of other dyes. The results, which were compared carefully, led us to rely chiefly on the two staining processes mentioned, but at the same time we often obtained valuable preparations with other staining reagents as well.

In spite of repeated attempts, we have not succeeded in observing the first nuclear division in the oogonium, but the later ones have been seen both in *Fucus vesiculosus* and in *F. platycarpus*, in which eight oospheres are formed. Oltmanns asserts that in *Ascophyllum*, in which only four oospheres are commonly formed, eight free nuclei occur at an earlier stage, but that four of these ultimately abort, and do not become centres of cell formation. Our observations tend to confirm him in this respect, but we found that in some cases a fifth oosphere, smaller than the rest, was occasionally differentiated, and that when freed from the oogonium it exerted an attraction on the antherozoids just like its larger sister oospheres.

When an oogonial nucleus is about to divide, it first becomes slightly, then very much, elongated so as to resemble an ellipse. Fine radiations are seen to extend from the two ends into the surrounding cytoplasm. The latter is at first tolerably uniformly granular, but as the radiations around the polar areas increase, these regions become cleared altogether of the granules which then become massed outside them. The nucleus rapidly becomes more spindle-shaped, and its chromatic elements are chiefly grouped near each pole, leaving a clear space about the equator in which the nucleolus is situated. In this respect the nuclei of *Fucus* offer a striking contrast to those of *Pellia epiphylla* already described (ANNALS OF BOTANY, vol. viii. p. 221) by one of us. In the latter plant the chromatic portion of the nucleus assumes an equatorial position at the corresponding stage in division, while the polar regions are clear.

The polar radiations continue to increase and the nucleus to lengthen, until the entire structure recalls the figure of a dumb-bell, in which the nucleus answers to the handle, and the radiation areas to the knobs. If the radii be traced outwardly, they are seen to terminate either in the frothy protoplasm, on the angles where the foam walls meet, or on the large granules which surround the cleared areas and are embedded in the foam. This point is one of considerable importance, and we shall revert to it further on. No structures were seen which could *certainly* be identified as centrosomes, although bodies suggestive of them were often observed; but these proved to be so variable in size and position, as well as in number, that we feel unable to attach any special significance to them.

The next stage in the mitosis is that in which the interpolar spindle arises, with the chromosomes disposed upon its equator. The spindle

is very remarkable inasmuch as it is entirely intranuclear, somewhat resembling that described by Fairchild for *Valonia*, or by Harper for *Peziza*. The nuclear wall can be distinguished until quite late in karyokinesis, and it is possible that no complete mingling of the cytoplasm with the contents of the nucleus takes place here. The spindle is extremely clear, and in several preparations, owing to a fortunate contraction during manipulation, the ends of the nuclear part of the spindle also had broken away from the cytoplasmic poles, and were visible as clean conical structures forming the poles of the nuclear spindle. The chromosomes were too minute to admit of their development being satisfactorily studied, but in *all the oogonial spindles* their number was estimated at *ten* when seen arrayed on the spindle equator. They were only seen in profile, and consequently it was difficult to be sure whether there were really ten or twelve, but the absolute number is not of importance as all the nuclei were compared from the same aspect. Remains, more or less preserving the original form, of the nucleolus were sometimes visible at this and even in a later stage. No division-planes are formed in the oogonium until the full complement of nuclei are produced; after this the positions which they will ultimately occupy are indicated by the heaping up into lines (or rather plates) of the cytoplasmic granules above referred to. These seem to be repelled equally from all the nuclei, thus effecting a symmetrical division of the entire oogonium.

After the complete delimitation of the oospheres within the oogonium, we observed, as an occasional circumstance, that one of the oospheres might contain two, or even three, nuclei, a fact also noticed by Oltmanns. When the oospheres are extruded, and come to lie free in the water, they grow in size, and are turbid with granules, which are very abundant in the cytoplasm. The chromatophores early become distinguishable from the other constituents of the cell, and the nucleus occupies a central position. It is itself surrounded by a dense layer of cytoplasm, which later on becomes very strongly marked. About five minutes after the mixing of the sexual cells, the antherozoids are found to have slipped into many of the oospheres. We failed to observe the act of penetration, but found a number of cases in which the antherozoid could be recognised within the oosphere, before its final fusion with the nucleus of the latter. It is a roundish, densely staining body, and, unlike the majority of animal sperm-cells as yet described, it imports into the egg no system of radiations along

with it. Judging from the short period of time elapsing between its penetration of the surface of the oosphere and its arrival at the exterior of the female nucleus, it must pass through the intervening cytoplasm with great rapidity. It then becomes closely appressed to the nucleus, and is about as large as the nucleolus of the latter. It rapidly spreads over a part of the female nucleus as a cap, and it presents a less homogeneous aspect than before. Both it and the female nucleus assume a granular condition, which is probably to be interpreted as representing a coiling and looping of the linin of the respective nuclei. Finally the two nuclei coalesce, and the original components can no longer be distinguished. Complete fusion may be effected in less than ten minutes after addition of the antherozoids to the water. The results are in striking accordance with those described by Wilson in connexion with the fertilisation of the eggs of Echinoderms in his recent Atlas of Fertilisation.

A delicate pellicle is meanwhile formed around the periphery of the oosphere, which is thus easily distinguished from the unfertilised oospheres, in which such a membrane in wanting. The texture of the cytoplasm also changes, and tends to assume a more definitely radiating character, the lines starting from the nucleus as a centre.

We observed, not unfrequently, rather large cells in which two nuclei of equal size were lying in close juxtaposition. These cells, with their nuclei, answer exactly to the description given by Behrens of the fertilisation stage in plants examined by him. We are unable however to accept his interpretation, for, in the first place, the series of fertilisation stages which we have observed, and have briefly described above, in no way correspond with the appearances described by him, and secondly, because these large cells (Behrens himself emphasises their size) are seen in material to which no antherozoids have had access. Furthermore, the average size of the young oospores is *not* obviously greater than that of the oospheres themselves. We regard the bodies in question as representing abnormal developments of oogonial cells, and not as being in any way concerned with fertilisation. Moreover, we have occasionally observed one cell in the divided oogonium much larger than the rest, to contain two, or even sometimes three, nuclei, and these nuclei are then always close together. These facts have led us to reject Behrens' account of the process.

A very large number of experiments were made, in order to determine, if possible, the time which elapsed between the addition of

the antherozoids to the oospheres and the first division of the spore. A short summary of different sets of observations on *Ascophyllum* is given in the subjoined tables.

SERIES I.—*Observations on* Ascophyllum *conducted at the Seaside.*

(*a*) The antherozoids were added to the oospheres at 10 o'clock A.M.

Lot 1.	Fixed 23 hours	after the addition	of antherozoids.		Nucleus preparing for division.
„ 2.	„ 24	„	„	„	Nucleus divided, rhizoid-rudiment present, no dividing wall.
„ 3 & 4	„ 32	„	„	„	Nucleus divided, no rhizoid, dividing wall present.
„ 5.	„ 36	„	„	„	Spore divided into about six cells.

(*b*) The antherozoids added between 11 and 12 P.M.

Lot. 1.	Fixed 24 hours	after the addition	of antherozoids.		Nucleus divided, a few with rhizoid-rudiments and division wall.
„ 2.	„ 25	„	„	„	Same result.
„ 3.	„ 25	„	„	„	Not beyond spindle stage.
„ 4.	„ 28	„	„	„	Nucleus divided, no rhizoid or dividing wall.

SERIES II.—*Observations on* Ascophyllum *carried on in the Laboratory.*

Antherozoids added between 5 and 7 P.M.

Lot 1.	Fixed 22½ hours	after the addition	of antherozoids.		Nucleus divided, no rhizoid or dividing wall.
„ 2.	„ 23	„	„	„	Nucleus preparing for division.
„ 3.	„ 23	„	„	„	Same as 1.
„ 4.	„ 24¾	„	„	„	Nucleus divided, rhizoid present, no dividing wall.

The above observations prove that there is no essential difference between the behaviour of material examined in London and at the seaside respectively.

After fertilisation, the cells rest for a long interval of time—commonly about twenty-four hours, as shown in the foregoing table—

before they begin to segment. The principal changes which occur during the interval are, first, in the rapid increase in the thickness of the peripheral cell wall, and, secondly, in the more regular arrangement of structure exhibited by the protoplasm. The alveolar, or foam character is extremely clear, and the chromatophores, which by this time have become very prominent, are noticed to be situated in the angles formed by the convergence of the foam walls; they are often bent and otherwise distorted, and so accommodate themselves to the structural condition of the foam. Other granules, which stain deeply, and probably represent food reserve of a proteid nature, are also abundantly scattered through the cytoplasm.

The first segmentation-division resembles, in a general way, the oogonial nuclear divisions already described, and the polar areas become similarly cleared of granules. The achromatic threads forming the polar radiations are very clearly seen to be attached to the foam-like structure of the cytoplasm, and indeed, in some cases, insensibly to pass into it. At other times fibrils end on granules (or, perhaps, on the protoplasmic lining of the granules), and sometimes again a fibril may fork, and its branches end either on granules or on the foam angles. The inference to be drawn from these facts seems to be that the radiations are the result of a change—a differentiation—in the protoplasm as it already exists, and that they do not owe their origin to the presence of any special 'spindle-forming substance,' by virtue of which they may be supposed to develop and 'grow' as new structures in the cell. We propose, however, to discuss the general bearings of our observations on this and on other questions of theoretical interest in a future memoir, in which the evidence for our views will be set forth in detail.

When the achromatic nuclear spindle appears, it also, as in the oogonial mitoses, is intranuclear, and it is often separated from the well-defined persistent nuclear wall by a clear space. The chromosomes, when assembled on the spindle, at the equator, are seen to be *twice as numerous* as in the oogonial nuclei, *i.e.* seen in profile we counted them as *twenty* in number. We were unable to distinguish any such grouping of the chromosomes as would lead to the conclusion that the chromosomes of the male and female nuclei respectively had so far preserved their original identity as to appear in the form of two separate groups. The long interval of time which, in *Fucus*, elapses between fertilisation and the first nuclear division possibly

may admit of a more thorough mingling or confusion of the parental chromosomes than would seem to be the case in some animals, *e.g.* the Copepoda as described by Rückert and by Häcker.

During the diaster stage the connecting achromatic fibres are at first very distinct, but they soon become fainter, and no cell-plate is formed across them. The two daughter nuclei generally pass into the state of rest, each being first hemispherical, with crenate projections on the flattened side turned towards its sister nucleus. Only after nuclear division is complete does the first cell wall appear. The cell is sometimes spherical when this happens, and then it is divided into two similar hemispheres. Further divisions may then appear, whilst the general contour of the embryo still remains more or less spherical. These cases occurred most frequently when the germinating spores were illuminated on all sides. But most commonly the first cell wall cuts the spore into two dissimilar halves, one of which grows out and forms a rhizoid. Often this projection is already apparent even before the first nuclear division occurs, and in any case one of the two daughter nuclei always passes down into the protuberance.

The immediately succeeding divisions have been sufficiently described by Thuret and others, but we may remark that the division of the nuclei in all cases precedes the formation of a cell plate, which is not formed in connexion with the achromatic connecting fibrils as in the higher plants.

The doubled number of the chromosomes is retained during the vegetative divisions of the thallus, and is constant throughout the somatic cells of the mature *Fucus* plant. Hence it follows that the reduction in the number of the chromosomes (in the female plants) is associated with the differentiation of the oogonium—the mother cell of the sexual products. Thus *Fucus*, in this respect, approximates more closely to the type of animal oogenesis than to that which obtains in those higher plants in which the details of chromosome reduction has been followed out.

Regarded from the standpoint of the number of its chromosomes, the *Fucus*-plant resembles the *sporophyte* of the higher plants, whilst the gametophyte of the latter, with its reduced number of chromosomes, finds its analogue merely in the maturing sexual cells of *Fucus*. But until we know more of the nuclear changes as they occur in other Algæ, and especially in the more primitive forms, it seems unadvisable

to go further than to indicate the possibility that we may require to revise our present ideas on the comparative morphology of the higher and lower groups of the vegetable kingdom. Even if we regard the reduction in the number of the chromosomes as a fact which is primarily of physiological importance, we may safely conclude, from the universality of its occurrence, that it is also intimately connected with the phylogenetic development of living forms, and hence it must meet with due recognition on the part of the morphologist who is engaged in comparing the life-history of one group of organisms with that of others.

The Development of Geothallus tuberosus, Campbell.

BY

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With Plates XXIV and XXV.

IN the spring of 1895 the writer received from San Diego specimens of a Liverwort which on examination proved to be so different from any described form, that it seemed necessary to establish a new genus to contain it. A preliminary account of the plant was therefore published[1], in which the name *Geothallus tuberosus* was proposed for it. The first lot of specimens were sterile; but later, through the kindness of Mrs. Katherine Brandegee, from whom the first lot had been received, fruiting plants were secured which proved conclusively that the plant represented a very low type of Hepatic, whose sporogonium was very much like that of *Sphaerocarpus*, a common Liverwort of the whole coast-region of California.

When first received, the plants had about completed their season's growth and were beginning to dry up. The thallus was partly buried in the light sandy earth in which they were growing, and they were quite overlooked at first. They were growing with plants of *Ophioglossum nudicaule*, and

[1] Campbell, A New Californian Liverwort, Botanical Gazette, Jan. 1896.

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when the latter were received they were placed under bell-jars to keep them fresh. The moisture started the Liverworts into growth, and their peculiar form at once attracted attention. When first seen, they were little fan-shaped fimbriated shoots, entirely different in appearance from any Liverwort with which the writer was acquainted. Further investigation revealed the presence of peculiar tubers, almost completely buried in the ground, from which these green shoots were evidently derived. A number of these were kept growing and developed imperfect reproductive organs, but these belated shoots did not develop in an entirely normal manner.

A considerable number of the tubers were allowed to dry in the earth where they had been growing, and were kept in this condition through the summer. In the autumn some of these were placed in water and germinated promptly. Repeated plantings were then made from time to time, and a good supply of normally developed plants was secured which made it possible to trace through the life-history of this interesting form with a fair degree of completeness, although a few points, notably the germination of the spores and the later stages of the embryogeny, were not as completely made out as might have been wished, owing to the limited amount of material. The results of these observations are given here, together with the conclusions based upon them as to the systematic position of *Geothallus*.

Germination of the Tubers.

The tubers, to which reference has already been made, constitute one of the most characteristic features of the plant. My attention has been called to a reference by Leitgeb[1] to what seem to be similar structures in the genus *Petalophyllum*, but no account was given of their structure or development. The thallus of *Petalophyllum*[2] is also said to be partially buried in sandy soil like that of *Geothallus*.

[1] Leitgeb, Untersuchungen über die Lebermoose, iii. p. 126.

[2] Schiffner, Hepaticae, in Engler and Prantl, Die Natürlichen Pflanzenfamilien, 91, 92, p. 59.

The ripe tuber in *Geothallus* is an oval, more or less flattened body, consisting of a mass of parenchyma densely filled with opaque granular contents, containing much fatty oil, but little starch. This mass of cells is surrounded by a layer of firm black cells, outside of which is a loose mass of dried-up parenchymatous tissue. A section of the ripe tuber (Fig. 1) shows at once that the bulk of it is derived from the central part of the thallus, and that the dark-coloured cells composing the inner rind are interrupted in front, where the growing-point of the thallus persists unchanged. The loose outer investment of the tuber is simply the remains of the leaves and the outer tissues of the thallus.

The anterior end of the tuber is free, and a longitudinal section shows that the apical cell of the shoot is still recognizable (Fig. 3, x). The regular segmentation is perfectly obvious, and is in no way different from that of the actively growing thallus. The apical cell and the younger segments cut off from it do not contain so much of the granular matter which renders the other cells of the tuber so opaque. The nuclei of the cells are of moderate size, but perfectly distinct and readily seen. The mass of cells making up the tuber is sharply marked from the outer tissues of the thallus by one or two layers of cells with thick black walls (Figs. 1, 2), and the growing-point is protected by the overlapping marginal lamellae or leaves with which the plant is provided. The full-grown tuber is from 1 to 2 mm. in length, and about half as wide.

The first experiments in germinating the tubers were made in September, and others were made at intervals during the autumn and winter. The tubers were taken from the earth, and after removing most of the dead tissue about them, were placed in a glass vessel with just enough water to cover them. The first lot of tubers was put in water on September 21, and on October 3 the green shoot was first seen. A second lot was placed in water on October 4, and on the 9th the young green shoots were already plainly visible. The best results and most vigorous plants were obtained from the germinations

made during October, November, and December, which is probably the natural period of germination. Those started later germinated less promptly, and in most cases failed to develop perfect reproductive organs. Where the tubers were placed in earth at once, they failed to germinate, at least early in the season, but what the explanation of this is it is difficult to see. It was found best to allow the tubers to remain in water until growth was well commenced, and then to transfer them to earthen saucers filled with sandy soil similar to that in which they originally grew. These saucers were placed in others filled with water and the top covered by a pane of glass. In this way abundant moisture was supplied, but evaporation was not prevented, as is the case under a bell-jar, and the plants grew much better than when grown in an absolutely saturated atmosphere.

As soon as the tuber is placed in water it absorbs the moisture rapidly, and the activity of the cells in the apex is almost at once resumed. Within two or three days the cells of the growing-point usually show more or less chlorophyll, which is quite absent from the cells of the ripe tuber. With the development of chlorophyll there begins an active growth in the apical region, which grows out into a green shoot which is very soon recognized as the first stage of the young thallus. This shoot is somewhat heart-shaped when seen from above (Fig. 5), and its margin is strongly fringed and lobed. From the lower cells numerous fine rhizoids with delicate, colourless cell-walls grow out, and, if the plant is now placed upon earth, quickly fasten the young thallus to the ground. As the thallus develops, the supply of reserve food in the tuber is rapidly exhausted, and the tuber finally decays. As it is very easily broken off it was overlooked when the plants were first discovered.

As the plant grows, there are formed from the margin structures which can only be designated leaves. These are formed in regular succession on either side of the growing-point, and recall strongly the similar organs in *Fossombronia* or *Petalophyllum*. These leaves are very conspicuous, espe-

cially when the plants are growing under glass, and give the plant an exceedingly characteristic appearance. In addition to the marginal lamellae or leaves, there are frequently formed similar outgrowths upon the dorsal surface, which, however, do not appear to bear any direct relation to the leaves, and are extremely variable in number, size, and position.

Apical Growth of the Thallus.

A vertical section through the apex of the thallus, at any stage, shows the free surface of the growing-point to be nearly vertical, and near the middle is the apical cell (Fig. 6, x), which is small when compared with the typical anacrogynous Jungermanniaceae, and recalls more nearly, perhaps, that of the Marchantiaceae. It is deeper, however, than is usual in those forms, or in *Sphaerocarpus*, which also has the apical cell of the same type and is not so near the ventral side of the thallus. Alternate dorsal and ventral segments are cut off from this cell in the usual way; but on account of the rapid secondary divisions in them, the limits of the segments are much sooner lost than in *Sphaerocarpus*. Some of the superficial cells of the vertical segments form jointed glandular hairs, like those commonly found in the anacrogynous Jungermanniaceae. They are much larger than the corresponding ones in *Sphaerocarpus*, and are not usually formed so near the apex. They lie on either side of the median line, so that an exact median section may fail to show them. Each hair (Fig. 9) consists usually of a small basal cell, and a row of several others, ranging from two or three to five or six in exceptional cases. The terminal cell is enlarged, somewhat pear-shaped in outline, and filled with densely granular contents. The secretion of this cell is probably mucilaginous, and the whole cell in the older hairs stains very strongly with Bismarck-brown. No doubt the mucilaginous secretion serves as a protection for the growing-point against evaporation.

In sections of the growing-point made horizontally, the apical cell appears four-sided in outline, often somewhat

narrower in front. It is not at all conspicuous, and is not always readily distinguishable from the adjacent segments. These are cut off from the lateral faces, apparently with considerable regularity, but their limits are soon lost. Comparing the horizontal section with the vertical section, it is evident that the form of the apical cell is that of a truncated wedge, from which four sets of segments are cut off, two lateral, one dorsal, and one ventral. It is in short exactly the same type as that of *Riccia* or *Sphaerocarpus*.

The Leaves.

The leaves arise as outgrowths of the superficial cells of the lateral segments, and apparently each segment gives rise to a leaf. They stand vertical at first, but later become more or less shifted, so that they are finally inserted obliquely. In horizontal sections of the growing-point (Fig. 7), the growing leaf (*l*) appears as a simple row of cells. If a surface-view of the leaf is examined, however, it is then seen that the leaf is roundish and slightly pointed in outline. In some cases at least there was evidence of definite apical growth from a two-sided cell, much like what obtains in the similar leaf-like lobes of the prothallium of *Equisetum maximum*. In other instances it looked as if the first wall of the mother-cell of the leaf bisected it and was followed by others at right angles to it (Fig. 8). In such cases there is probably no definite apical growth. As the leaf enlarges, the marginal growth is unequal, and the older leaves are often decidedly irregular in outline and deeply lobed, so that their original arrangement is obscured. The development of leaf-like outgrowths from the dorsal side of the thallus, which are often partially confluent with the true leaves, disguise still further the two-ranked arrangement of the latter. These dorsal lamellae, as well as the leaves themselves, are for the most part but a single cell in thickness, but the base is usually thicker, merging somewhat gradually into the body of the thallus.

In the original specimens sent from San Diego, the thallus

was more or less wedge-shaped, but often nearly orbicular in outline. The plants were growing crowded together, and almost buried in the earth, and as they were beginning to dry up were not at all conspicuous and, as already said, were quite overlooked at first, and it was not until the moisture with which they were supplied started them into growth that attention was called to them. These plants were about 5 to 7 mm. in length by 3 to 4 mm. in breadth, and often quite unbranched, or only once dichotomous. The plants raised in the laboratory were usually somewhat larger than the original ones, and branched more freely. This greater vigour, as well as the more luxuriant development of the leaves, was no doubt owing to the great amount of moisture furnished them. The branching in all cases was a typical dichotomy like that found regularly in the thallose Liverworts.

In connexion with the freer branching and better development of the leaves of the plants grown under glass may be mentioned a similar acceleration of growth in *Sphaerocarpus*. A number of specimens of *Sphaerocarpus terrestris var. californicus Aust.* appeared in one of the cultures of *Geothallus*, and these differed so much from the normal plants as to be scarcely recognizable. They grew very vigorously and decidedly exceeded the normal plants in size. This was especially true with the male plants, which were several times larger than is usually the case. They branched freely, and developed unmistakable leaves, not unlike those of *Geothallus*, while the normal plants have the margin of the thallus quite undivided [1].

The thallus of *Geothallus* is fastened to the earth by very numerous white rhizoids. These are outgrowths of superficial cells, and are thin-walled and colourless like those of most Jungermanniaceae.

In regard to the arrangement of the leaves, *Geothallus* resembles most nearly *Fossombronia* among the American

[1] For particulars concerning these abnormal specimens of *Sphaerocarpus*, see a recent article by the writer in Erythea, May, 1896.

genera of Liverworts. Some of the specimens examined strongly recalled *F. longiseta*, a common and characteristic Californian species. From *Petalophyllum*, with which *Geothallus* seems to agree in the general form of the thallus and arrangement of the leaves, it differs essentially in the absence of anything resembling the anterior leafless prolongation of the thallus described and figured by Leitgeb [1]. Unfortunately this genus does not occur in the United States, so there was no opportunity of examining the plants themselves for purpose of comparison.

Under favourable conditions the sexual organs were mature within a month from the time the dry tubers were placed in water. Those started in the spring and the first ones started in the autumn did not produce normal reproductive organs, although these began to form. Most of those started after January 1 also failed to mature the sexual organs, and in some of the plants grown from these belated plantings the reproductive organs were almost entirely wanting. *Geothallus* is dioecious, but there is not much difference in the size and general appearance of the male and female plants, in which respect it offers a strong contrast to *Sphaerocarpus*, where the male plants are very much smaller than the female.

In their position and general structure the sexual organs resemble those of *Sphaerocarpus*, and each archegonium or antheridium is surrounded by a separate envelope, that about the archegonium becoming very conspicuous. As a rule the sexual organs are produced less abundantly than in *Sphaerocarpus*, but are decidedly larger. The time of their first appearance varies a great deal, in some cases being evident when the young shoot was not more than a millimeter in length; but in most cases the young thallus had reached a length of several millimeters before the youngest ones were to be detected. Where the development of the sexual organs was partly suppressed, the envelope was not usually fully developed, and projected but slightly above the surface of the thallus, recalling the simple pores of many Marchantiaceae.

[1] Leitgeb, l. c., iii. p. 126, Pl. IX, Figs. 16–19.

THE ANTHERIDIUM.

There is a considerable variation in the form and size of the male plant, as well as in the number of antheridia produced. The thallus may remain unbranched (Fig. 10), or it may fork once or twice. No definite relation between the young antheridium and the segments of the apical cell could be discovered. As the young antheridia seldom stand exactly in the median line of the thallus, and are usually more or less inclined forward, exact median sections are not always easy to get; but by cutting the thallus at different angles it was possible to obtain a series of slides which showed most of the different stages of development.

The antheridium mother-cell (Fig. 11) is very early distinguishable from the neighbouring superficial cells of the thallus, and is formed further back from the apex than is the case in *Sphaerocarpus*[1]. It is an elongated cell which projects strongly above the adjacent cells, and its nucleus is decidedly larger than the nuclei of the neighbouring cells. The protoplasm is also denser, but the cell contains several large vacuoles. The first division-wall is horizontal, and nearly on a level with the surface of the thallus. This is followed by a similar division in the upper cell, dividing it into two of unequal size (Fig. 12). The original cell is thus divided into three superimposed cells, of which the basal one is coherent with the adjacent cells of the thallus, and the upper ones are free. Of the two upper cells, the lower smaller one forms the pedicel of the antheridium, the other its body. These first divisions correspond exactly to those in *Sphaerocarpus*, and also in some other Liverworts. The basal cell divides usually by intersecting vertical walls, and forms a group of about four cells at the base of the antheridium, but takes no further part in its development.

The subsequent divisions in the antheridium show considerable variation, and one of much interest in connexion

[1] Campbell, Mosses and Ferns, p. 79, Fig. 31.

with the question of the affinities of *Geothallus*. The first division in the upper cell, instead of being transverse as in *Sphaerocarpus*, is vertical or oblique, agreeing thus with the typical Jungermanniaceae. In case the first wall is vertical, it is followed by two horizontal walls (Fig. 13), and each of the four cells is again divided by vertical walls so that regular octants are formed as in *Sphaerocarpus*. Where the first wall is strongly inclined (Fig. 14), the secondary wall is found first in the larger of the two cells, and the effect is produced of a two-sided apical cell like that found in the typical Moss-antheridium. A variation of this kind has been noted by the writer in *Riccia*[1]. Whether in any of these instances the regular octant-formation was suppressed could not be certainly determined; but certain irregularities noticed in the cross-sections of the antheridium would indicate that this probably is sometimes the case.

The next divisions are generally periclinal, and effect the separation of the central mass of cells, from which later the sperm-cells develop (Figs. 15, 16). These central cells soon acquire very dense contents, which stain strongly, and divide rapidly until the full number of sperm-cells is formed. These cells are arranged in groups corresponding to the primary divisions in the antheridium, and, especially where the contents have been slightly contracted through reagents (Fig. 17), show these primary divisions for a long time. The divisions occur mostly at right angles to each other, and the resulting sperm-cells, as so frequently happens in the Bryophytes, are nearly cubical. The nucleus of the sperm-cells is relatively large, but the sperm-cells themselves are smaller than is usual in the Jungermanniaceae. In this respect, and in the consequent small size of the spermatozoids, *Geothallus* recalls *Riccia*. Owing to their very small size, the spermatozoids do not offer a good subject for studying their development, and no attempt was made to follow this out. There was nothing to indicate any departure from the ordinary process. The free sperma-

[1] Campbell, Mosses and Ferns, p. 33.

tozoids (Fig. 20) are extremely small, and show about two coils. The tapering forward end is provided with the usual two cilia, which are almost equal in length to the body of the spermatozoid.

The stalk of the antheridium is derived from the second of the three primary cells into which the mother-cell is divided. This cell is often divided by cross-walls, so that the stalk is composed of four short rows of cells; but quite as often the second vertical division is suppressed, and it is then made of but three (Fig. 15, *a*). The transverse divisions are never numerous, and the pedicel remains short. In the formation of longitudinal walls in the pedicel *Geothallus* differs from *Sphaerocarpus*, where the pedicel always is composed of but a single row of cells, and resembles much more *Fossombronia* or *Pallavicinia*[1], where there are also found quadrant-walls in the body of the antheridium. At maturity it is usually somewhat oblique, being bent forward more or less.

Each antheridium is surrounded by an envelope, closely resembling that found in *Sphaerocarpus*, and formed in exactly the same way. Almost simultaneously with the first division in the antheridium mother-cell, the superficial cells immediately begin to grow out about it in the form of a ring-shaped wall, and very soon outstrip the antheridium, which is sunk in the involucre thus formed. The wall of the involucre remains but one cell thick, except at the extreme lower part where it joins the thallus. The upper part of the envelope is prolonged into a neck, through which the spermatozoids are discharged. The enlarged lower part fits closely over the antheridium (Fig. 19).

Sometimes the antheridia are produced only in small numbers, and may form a single row occupying the median line of the shoot; but usually they are produced in larger numbers, and may almost completely cover the dorsal surface of the thallus (Fig. 10), much as in *Sphaerocarpus*.

[1] Leitgeb. l. c., iii. p. 88.

In a few cases two antheridia were seen within the same envelope. They were strongly flattened on the side where they were in contact, but there was no means of deciding whether or not they originated from a common mother-cell.

The Archegonium.

The female plant does not differ much from the male, but is possibly a little larger, as a general thing, and more freely branched (Figs. 21, 22). Like the antheridia, the archegonia arise from superficial cells near the apex of the shoot, and at first are hardly distinguishable in form from the youngest antheridia. The mother-cell of the archegonium, however, is somewhat larger and more enlarged above. The first wall corresponds to that in the young antheridium, and is nearly level with the surface of the thallus. The second wall is also horizontal, as in the antheridium, but it is formed in the lower of the two primary cells, instead of in the upper one, which becomes at once the archegonium proper, the two lower cells forming the short pedicel (Fig. 24). The form of the mother-cell of the archegonium, as well as the first division, correspond very closely with the same points in *Sphaerocarpus*, the greatest difference being the position of the first wall, which is higher up in *Sphaerocarpus*, so that later the archegonium is raised above the level of the thallus. The first divisions in the upper cell are those typical of the Liverworts in general. The usual three intersecting walls arise, cutting off the axial cell (Fig. 25), from which later the egg-cell, canal-cells, and cover-cell are developed. Then each of the primary peripheral cells is bisected by a second vertical wall, after which by horizontal walls in all the cells the archegonium-rudiment is divided into two tiers, constituting respectively the venter and neck. Before this takes place, however, the cover-cell has been cut off from the axial cell. The archegonium now (Fig. 26) consists of six peripheral rows of cells enclosing an axial row of three cells. The cover-cell next undergoes division into four by cross-walls, and the two

inner cells by transverse divisions give rise to the egg and the row of canal-cells. The central cells have larger nuclei and denser protoplasm than the peripheral ones. In one case (Fig. 29) apparently two ventral canal-cells had been cut off successively, and some older ones showed what seemed to be evidences of the same thing. The writer has in a former paper called attention to a very similar phenomenon observed in *Osmunda*[1]. What the significance of this is would be hard to conjecture. The suggestion made with reference to *Osmunda*, that possibly it was something analogous to the polar-body of the animal ovum, seems hardly in accordance with the phenomena of fertilization known in plants.

The neck-canal-cell divides first into two (Fig. 27), and later each of these divides once more. The wall of the archegonium remains single-layered until after it is fertilized. A cross-section of the neck of the archegonium shows six peripheral cells, as in *Sphaerocarpus* and the Marchantiaceae, instead of only five, the number in the typical Jungermanniaceae. The basal cells undergo divisions in various directions, so that the pedicel is not clearly distinguishable in the older archegonium. The venter of the ripe archegonium is not much enlarged, and the elongated egg does not completely fill its large cavity (Fig. 31).

As in *Sphaerocarpus*, the envelope is formed about the archegonium whether it is fertilized or not; but in *Geothallus* it grows more slowly at first, and at the time the archegonium opens it does not reach more than about halfway up the neck, the upper part of which projects out of the opening. Later, however, it continues to grow until it finally becomes very conspicuous (Fig. 23). Its form is decidedly more cylindrical than in *Sphaerocarpus*, and the opening at the top larger. As in *Sphaerocarpus*, the neck of the archegonium is more or less bent forward.

[1] 'On the Prothallium and Embryo of *Osmunda*,' Annals of Botany, April, 1892.

EMBRYOGENY.

The fertilization of the egg was not studied, as the extremely small size of the spermatozoid did not promise satisfactory results. After fertilization the egg increases in size, and the granular contents become less conspicuous. The primary division (basal wall) is transverse, and divides the egg, which still retains its elongated shape, into two cells of nearly equal height, but the lower one is more tapering than the nearly hemispherical upper one. This first division determines the separation of the capsule from the stalk, as in the Marchantiaceae and *Sphaerocarpus*. The primary wall is followed by a similar one in each cell, so that the embryo (Fig. 33) consists of a row of four, resembling very closely, as it does in the subsequent stages, the corresponding stages of *Sphaerocarpus*. The lower of the two cells derived from the hypobasal half of the embryo undergoes no further division, but remains unchanged and easily recognizable for a long time (Fig. 35). Vertical walls now arise in all of the upper cells, which usually, at least, divide each of them into four nearly equal quadrant-cells (Fig. 34). Somewhat later (Figs. 35, 36) the upper part of the embryo enlarges more rapidly, and a series of periclinal walls is formed separating a central group of cells, the archesporium, from a single layer of peripheral ones which undergo no further periclinal divisions, but persist as the wall of the capsule. In the hypobasal cells the divisions are less regular, but here, too, there is unequal growth, the central part of the embryo remaining narrow, while the lower cells, by repeated division and increase in size, form a conspicuous nearly globular foot.

Owing to the small number of embryos available for study, it was not possible to determine with certainty whether the succession of the divisions in the young embryo given above is always exactly the same. Thus it is possible that a part of the short seta may arise from the epibasal portion of the embryo, as in the typical Jungermanniaceae, but there seems to be no doubt as to the hypobasal origin of the foot. Inter-

mediate stages between those shown in Fig. 35 and Fig. 37 were wanting, so that the important point of the origin of the sporogenous and sterile cells must for the present remain undecided. A comparison of the stage shown in Fig. 37 with the corresponding one in *Sphaerocarpus* shows some marked differences. While the structure of the wall is the same, the wide space between the mass of archesporial cells and the wall in *Sphaerocarpus* is absent here, and the sporogenous cells are very much larger and entirely free, instead of being united with the sterile cells, from which they also differ much more in appearance than is the case in *Sphaerocarpus*. The spore-mother-cells in the specimen figured (Fig. 38, *a*) were free globular cells with a thick membrane which appeared quite homogeneous in structure. The centrally-placed nucleus was not very large, nor was the amount of chromatin especially noticeable. In the microtome-sections the cytoplasm showed a reticulate appearance like that observed in other similar cells, and due no doubt to the dissolving out of the oil or other soluble matter by the reagents employed in the process of imbedding. The sterile cells (Fig. 38, *b*) are thin-walled and almost transparent, and entirely separated from each other. They contain very little granular matter, but the nucleus is distinct.

No specimens were secured which showed the early stages in the division of the spores, and the appearance of the older spore-tetrads does not show whether or not there is any indication of the division of the cell before the nucleus divides.

The Mature Sporogonium [1].

The mature sporogonium is a nearly spherical capsule, about 1 mm. in diameter, connected by the very short seta, which is about four cells thick, with the enlarged bulbous foot which penetrates into the thallus, instead of being raised above it as

[1] For figures of the mature sporogonium and spores, see the writer's paper in the Botanical Gazette, January, 1896.

in *Sphaerocarpus*. This is due to the more nearly sessile position of the archegonium in *Geothallus*. The wall of the capsule is composed of a single layer of large almost black cells, which before it ripens are filled with starch which mostly disappears later. The foot is globular or sometimes oval in outline, and its cells much distended and containing abundant protoplasm and large nuclei, showing that they are actively engaged in the nutrition of the sporogonium.

The spores are very large, and at maturity separate completely. They are nearly globular and range from 120–140 μ in diameter. The wall is very thick, and in section shows two well-marked layers, the perinium and the exospore. An endospore of cellulose is probably present, but in microtome-sections is not clearly differentiated from the inner layers of the exospore. The perinium is almost black and appears perfectly homogeneous: it is quite smooth except upon the ventral surface of the spore, where it is folded so as to produce reticulate ridges which in section have the appearance of spines. These foldings extend to the inner spore-coats as well. No chlorophyll is present in the ripe spore, but no further study of the spore-contents was made beyond noting that the nucleus is small, as is usually the case in Liverworts.

The sterile cells, which doubtless are the homologues of the elaters of the more specialized of the Hepaticae, reach a length of 48–108 μ, being relatively longer than the corresponding cells of *Sphaerocarpus*. They contain some chlorophyll and a few scattered granules apparently of albuminous nature, but little or no starch was detected in any of the specimens examined.

The sporogonium does not break through the calyptra until a much later period than in *Sphaerocarpus*, and traces of the calyptra remain until the sporogonium is almost ripe. The first divisions in the cells of the venter coincide pretty closely with the first wall in the embryo. Except at the base it remains two cells thick for the most part. Finally it is torn asunder by the expanding sporogium, and the upper

part, with the neck of the archegonium, is carried up on the apex of the capsule. The basal part remains as a sheath surrounding the short seta, while the foot is completely sunk in the thallus.

Formation of the Tubers.

The first indication of the formation of the tubers in the female plants is evident almost as soon as the first archegonium is fertilized; but in the shoots where no perfect sexual organs were developed, and in the male plants, it is of course independent of fertilization. It begins by the accumulation of granular matter in a group of interior cells near the growing-point, which soon is very evident in section as an opaque area of varying size. This area rapidly spreads until it occupies the greater part of the axial tissue of the shoot, and its limits finally become very sharply defined. Later the chlorophyll disappears completely from these cells, and those which immediately surround the developing tuber have their walls thickened and form the rind. We have seen, however, that this rind does not extend over the growing-point itself, whose apical cell and the immediately adjacent tissue remain practically unchanged, and ready to resume active growth again when the conditions are favourable. The leaves and outer tissues of the thallus finally die, and in the fruiting plants the spores are set free by the decay of the walls of the capsule.

Germination of the Spores.

A small number of spores sown in October germinated promptly, and some of the earlier stages were seen; but another sowing made later was unsuccessful, so that the account here given is necessarily very incomplete. A very long germ-tube was formed in all the cases observed (Fig. 39), and into this the granular contents, largely in the form of oil-drops, pass. Very little chlorophyll is present at first, but this rapidly increases in amount. The end of the germ-tube, where the granular protoplasm becomes much denser, is cut

off by a transverse wall. The next divisions were not seen, and in the youngest stage, found subsequently, there was a large two-sided apical cell present, which looked as if it might have been formed by the intersection of the first two walls in the terminal cell (Fig. 40). It is very probable that there is no absolute uniformity in the divisions, and that octant-divisions, such as occur frequently in most of the Marchantiaceae and *Anthoceros* as well as in *Sphaerocarpus*, may also be found. In the later stages the basal part of the thallus had assumed a cylindrical form, while the end was flattened out into a lamina but one cell thick. In Fig. 41 is shown one of these with a cell (x) which may perhaps be the apical cell, although its lateral position might be against this. Since, however, in the still older plants studied, the growing-point was strongly lateral, this objection is not a valid one. A most striking feature of the older plants (Fig. 42) was the presence of the leaf-like lobes which were found on either side of the growing-point and gave the thallus a most characteristic appearance, very different from the corresponding stage of *Sphaerocarpus* or the Marchantiaceae, and resembling more the young thallus of *Anthoceros fusiformis*, or still more the prothallium of *Equisetum*. Just at what point the two-sided apical cell of the young thallus is replaced by the form characteristic of the older one could not be determined. In none of the specimens examined was a rhizoid developed from the base of the germ-tube, but the first one grew out from one of the basal cells of the thallus itself (Fig. 42).

Summary and Conclusions.

There is little doubt that on the whole *Geothallus* agrees more nearly with *Sphaerocarpus* in its structure than with any other known form, and may very properly be placed in the same family, which also includes, according to Schiffner [1], the imperfectly known *Thallocarpus*. Leitgeb [2] unites *Riella*

[1] The Hepaticae, in Engler and Prantl, Die Natürlichen Pflanzenfamilien, 91, 92, p. 50.

[2] Leitgeb, l. c., iv. p. 9.

with *Sphaerocarpus* into the family Rielleae, but Schiffner[1] makes a special family, Rielloideae, to include the genus *Riella*. The four genera all agree in the absence of perfect elaters, which are replaced by the thin-walled chlorophyll-bearing cells, and constitute the lowest group of the anacrogynous Jungermanniaceae, the Anelatereae.

Geothallus agrees with *Sphaerocarpus* in the form of the apical cell, the general position and structure of the sexual organs, including the characteristic envelope with which each is surrounded. In all these particulars both genera resemble *Riccia*, where the transverse primary divisions in the antheridium, as well as the subsequent quadrant-divisions in the upper cells, are quite different from the corresponding divisions in the antheridium of the typical Jungermanniaceae. The six outer rows of cells in the neck of the archegonium in both genera are also suggestive of the Marchantiaceae rather than of the typical Jungermanniaceae, where there are but five. But the most important point of resemblance between *Geothallus* and *Sphaerocarpus* is the sporogonium, in both the structure and development of which they agree very closely.

The most important points in which *Geothallus* differs from *Sphaerocarpus* are—its much more massive thallus; the second division in the antheridium and the massive stalk of this organ; the sessile archegonium, and consequent deeper penetration of the foot of the embryo into the thallus; the large size and complete separation of the smooth spores; and, most important, the development of true leaves and the formation of tubers by which the plant becomes perennial. It will be remembered, however, that under certain conditions *Sphaerocarpus* may also develop leaf-like organs.

The points of resemblance with *Riccia* have already been mentioned. Among the typical anacrogynous Jungermanniaceae, *Fossombronia*, at least of the American genera, resembles *Geothallus* most closely. It differs, however, in several important particulars, apart from the very much more

[1] Schiffner, l. c., p. 51.

highly developed sporogonium with its long seta and true elaters. The growth of the thallus is from a two-sided apical cell; the sexual organs are destitute of a special envelope; the divisions in the antheridium are those of the typical Jungermanniaceae; and the neck of the archegonium has five peripheral rows of cells.

Petalophyllum, according to Leitgeb [1], agrees closely with *Fossombronia* in its apical growth and in the development of its leaves. The antheridia are partially surrounded by an imperfect envelope, but the archegonia are in groups. An interesting resemblance between *Petalophyllum* and *Geothallus* is the formation in the former of bulb-like structures, probably similar in nature to the tubers of *Geothallus*. This is associated with the burying of the thallus in the sandy soil where the plant grows [2].

All of the Anelatereae show unmistakable resemblances to the lower Marchantiaceae, especially *Riccia*, and are probably pretty near the point at which the Marchantiaceae and Jungermanniaceae diverge. *Sphaerocarpus* is on the whole the most primitive type, and *Geothallus* may be said to be an intermediate between it and forms like *Fossombronia*. This is indicated both by the structure of the antheridium and the leaves. The development of leaves, however, cannot be considered as being very important as bearing upon its relationship with other forms, since this has occurred beyond question at several points in the series of Anacrogynae. It emphasizes, however, the exceedingly generalized and presumably primitive character of the whole group, and its importance in a study of the origin of the higher Archegoniates.

[1] Leitgeb, l. c., iii. p. 130.

[2] Ibid. p. 126, footnote.

EXPLANATION OF FIGURES IN PLATES XXIV AND XXV.

Illustrating Professor Campbell's paper on *Geothallus tuberosus*.

PLATE XXIV.

Except where otherwise stated, the drawings were made from microtome-sections of material fixed with chromic acid or absolute alcohol.

Fig. 1. Nearly median section of the old thallus of *Geothallus tuberosus*, showing the tuber, t. × 20.

Fig. 2. The hinder part of the tuber, × 300.

Fig. 3. Forward part of the tuber surrounded by the overlapping leaves, and showing the apical cell, × ; × 300.

Figs. 4, 5. A germinating tuber; *a*, from the side; *b*, from above; × 20. *k*, the young shoot.

Fig. 6. Vertical section of the growing-point of a young shoot, × 300. ×, the apical cell; *l*, leaves.

Fig. 7. Horizontal section of a similar apex, × 300. *h*, ventral hairs.

Fig. 8. Young leaf, × 300. *D*, dorsal; *V*, ventral margin.

Fig. 9. Glandular hair from the ventral surface, × 300.

Fig. 10. Male plant, with numerous antheridia, × 20.

Figs. 11–16. Successive stages in the development of the antheridium, × 600. Fig. 15, cross-sections, the others longitudinal. In Fig. 15, *a* is a section through the stalk, *b* through the body of the antheridium.

Fig. 17. An older antheridium, median longitudinal section, × 300.

Fig. 18. Surface-view of the cells from the wall of the older antheridium, × 300.

PLATE XXV.

Fig. 19. Median longitudinal section of a nearly ripe living antheridium, showing the form of the envelope, × about 100.

Fig. 20. Spermatozoids killed with osmic acid, × 1000.

Fig. 21. Female plant, × 5.

Fig. 22. End of one of the branches of the same, × 20.

Fig. 23. Section of an older female plant, showing the archegonal envelopes (♀), × 5.

Figs. 24–26. Young archegonia, median longitudinal sections, × 600.

Figs. 27, 28. Two longitudinal sections of an older archegonium, × 300. The egg and ventral canal-cell only are shown in Fig. 28.

Fig. 29. Venter of an archegonium, with two ventral canal-cells, V^1, V^2, × 300.

Fig. 30. Longitudinal section of an open but unfertilized archegonium, × 300.

Fig. 31. Venter of an open archegonium, showing the recently fertilized egg, × 300.

Fig. 32. Cross-section of the neck of the archegonium, × 300.

Fig. 33. A four-celled embryo, enclosed in the venter of the archegonium, × 300.

Fig. 34. Longitudinal section of an older embryo, × 300.

Fig. 35. Similar section of an older embryo, showing the calyptra, × 300.

Fig. 36. Two transverse sections of an embryo of about the same age as that shown in Fig. 35. × 300. *a* is the apical region; *b* is lower down.

Fig. 37. A section through the upper part of a young sporogonium, after the separation of the spore-mother-cells, × 50.

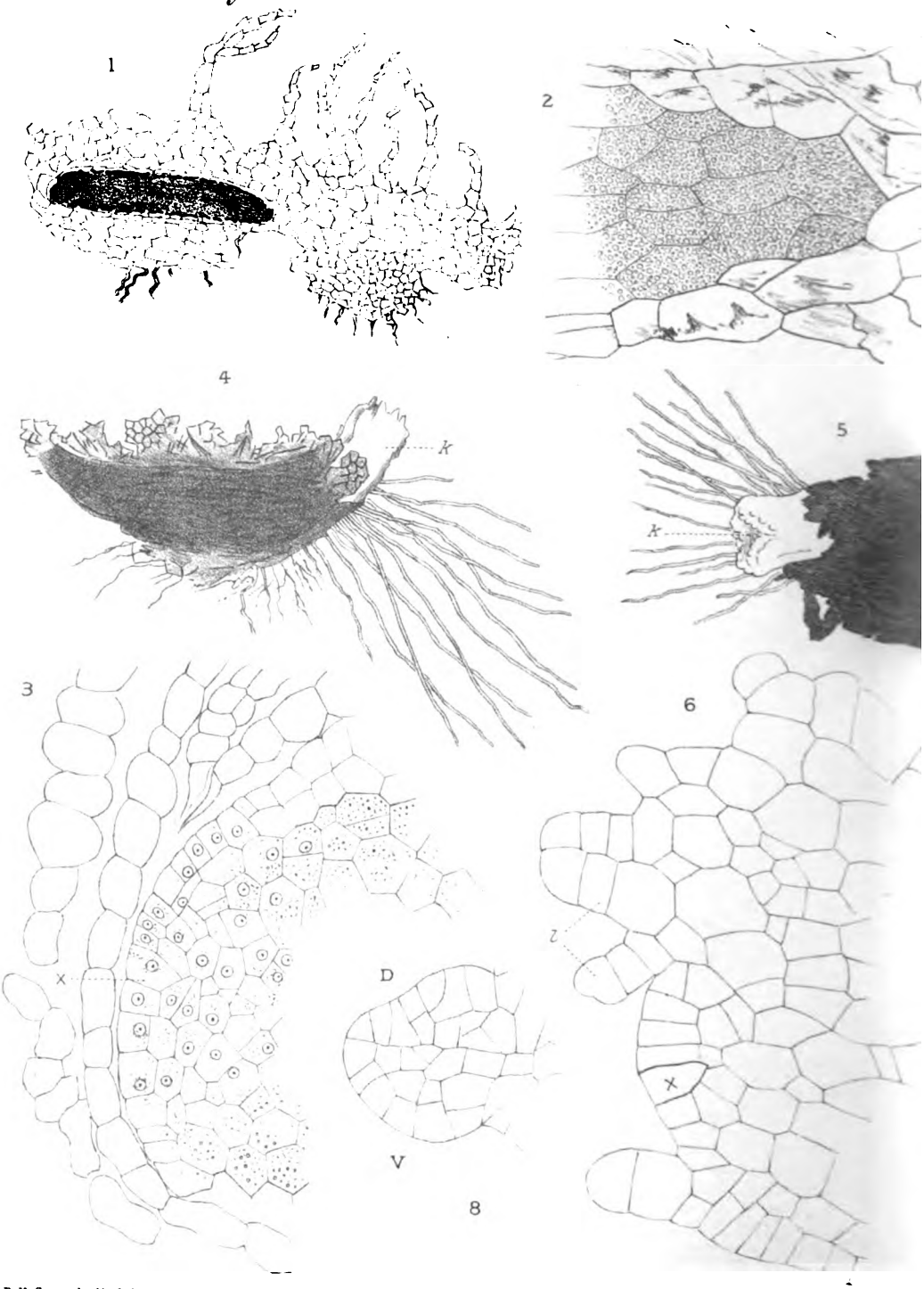
Fig. 38. A spore-mother-cell and two sterile cells from the same sporogonium, × 300.

Fig. 39. A germinating spore, × 100.

Fig. 40. Apex of very young thallus, showing the two-sided apical cell, ×; *g*, the germ-tube; × 300.

Fig. 41. A somewhat older stage, × 300.

Fig. 42. Young plant still showing the germ-tube, *g*, and spore, *sp*, × 40. ×, the growing-point of the thallus.



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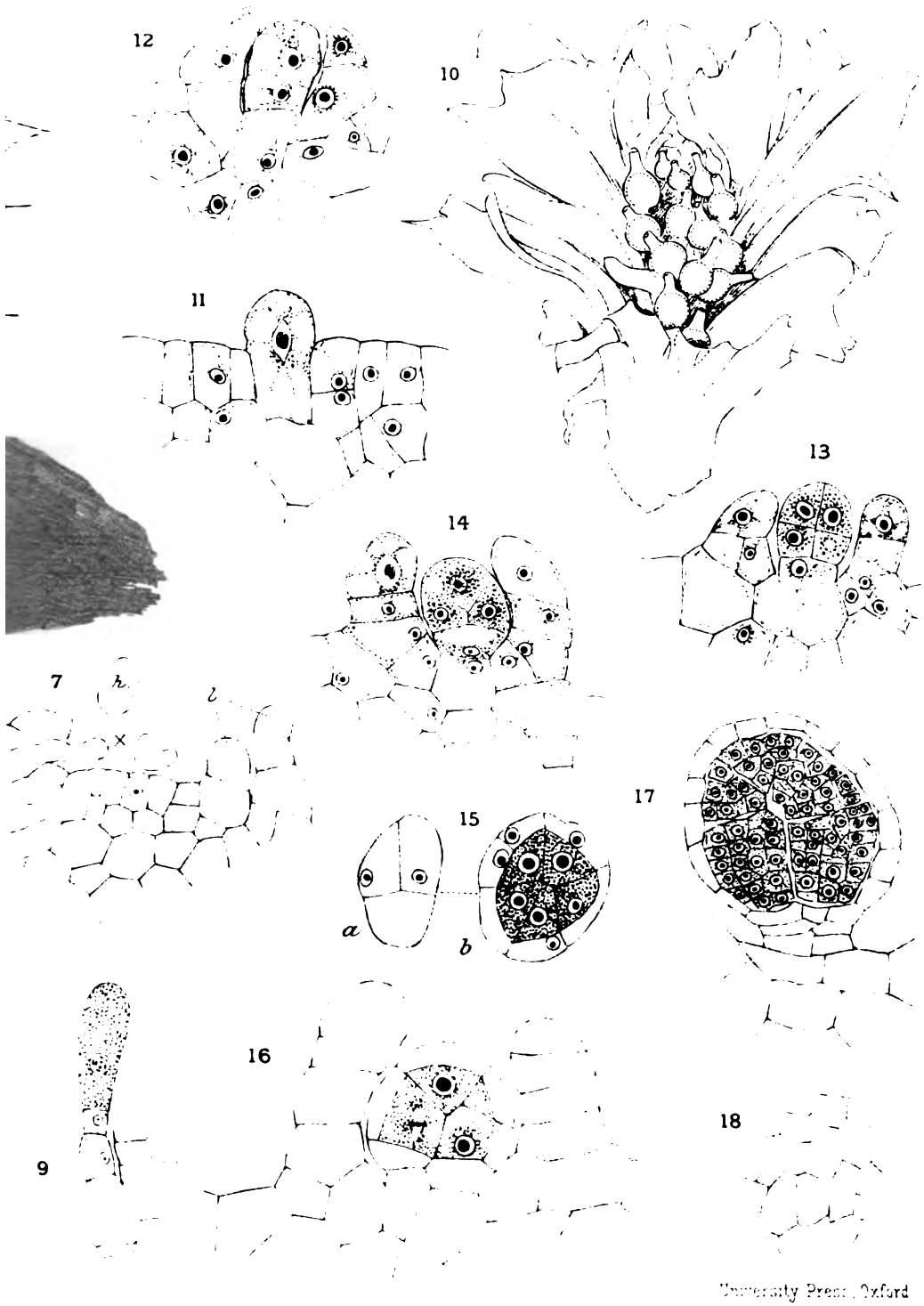

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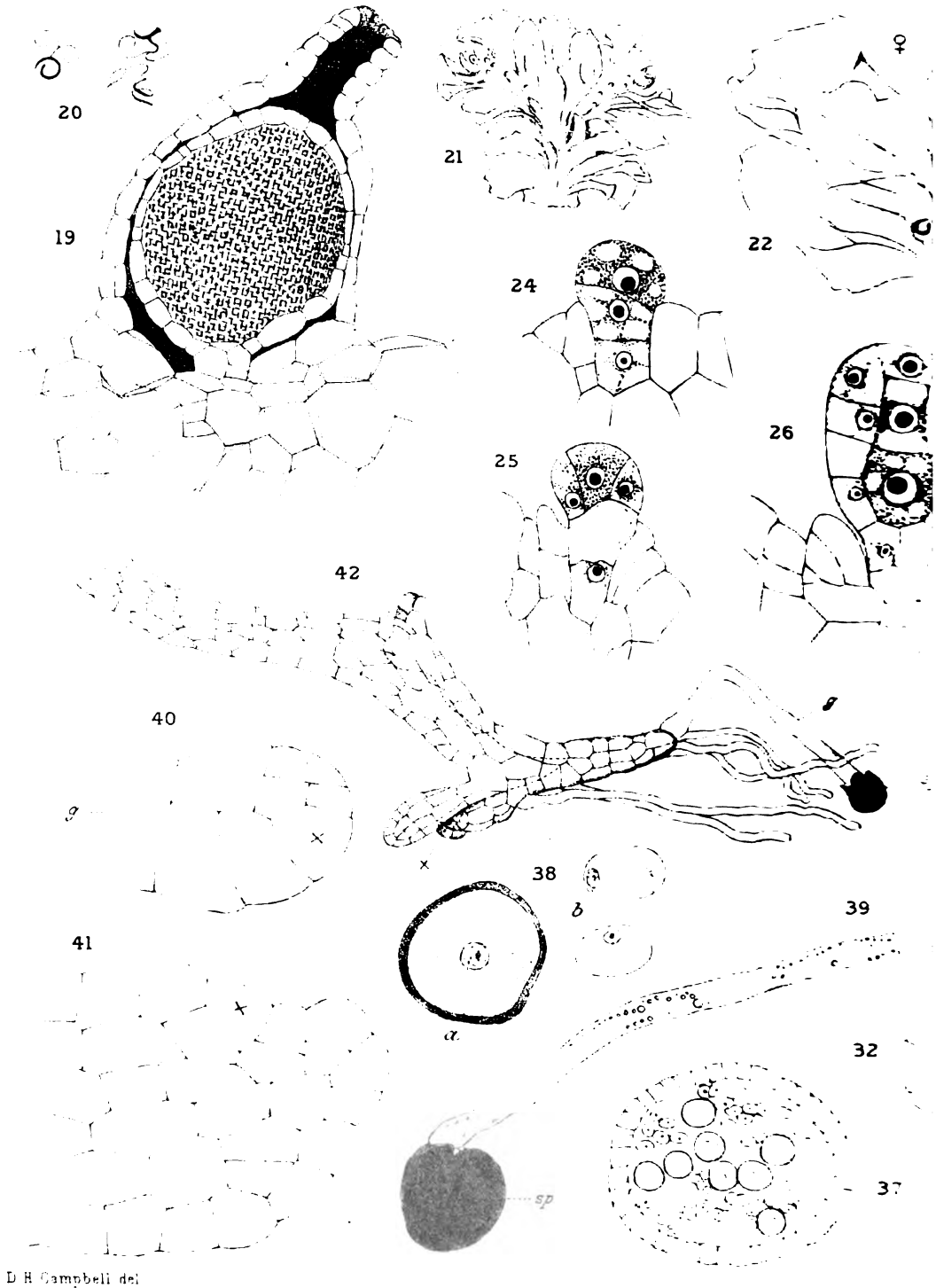

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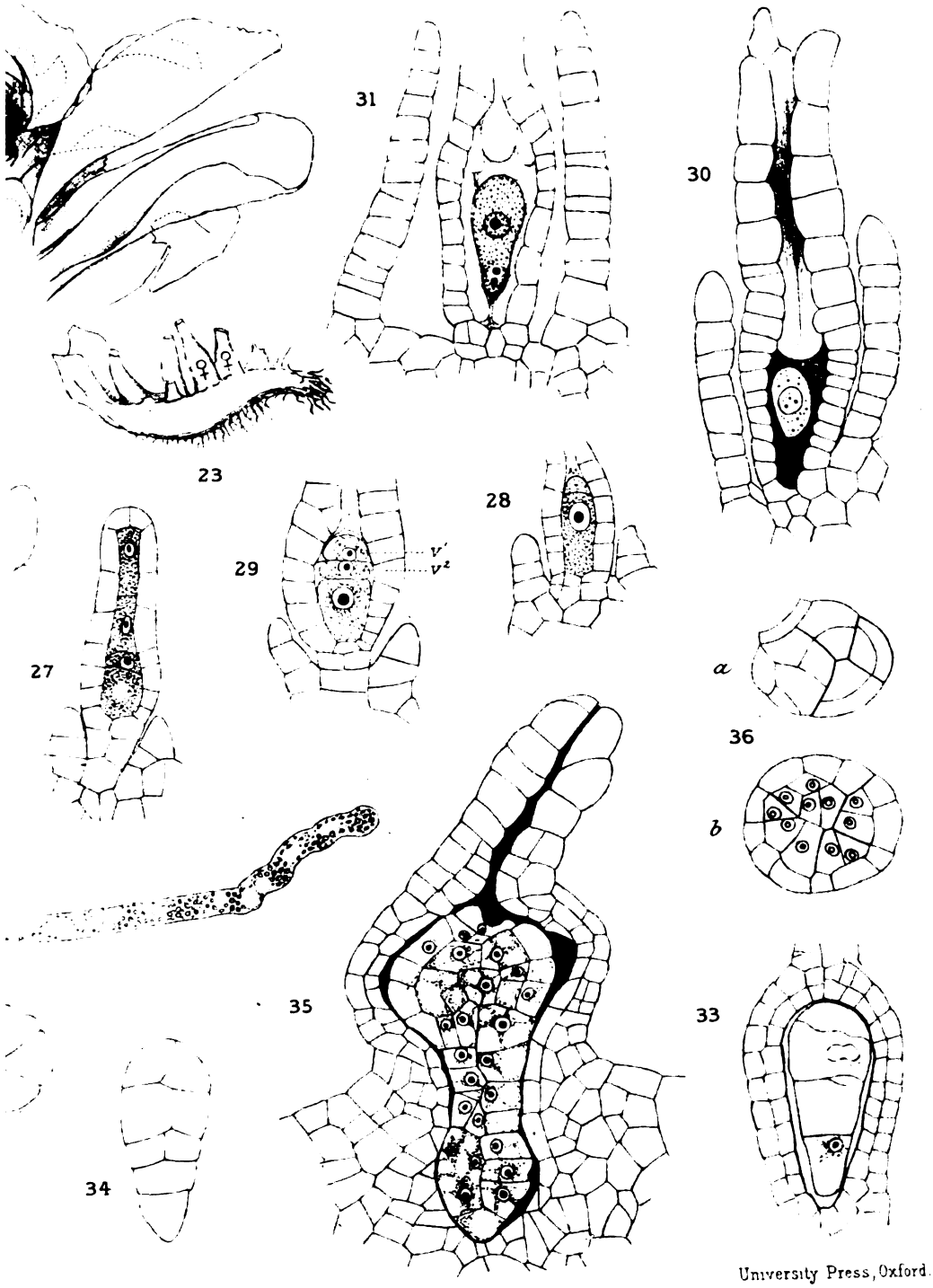

University Press, Oxford.

The Influence of Fruit-bearing on the Development of Mechanical Tissue in some Fruit-trees[1].

BY

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HISTORICAL.

THE attention of botanists appears to have been first drawn to the difference between the anatomical structure of the flower-stalk and that of the leafy stem by the work of Oels in 1879[2]. Oels studied the Droseraceae, and found little difference between the structure of the axis of inflorescence and that of the leafy stem.

In a paper published in 1886, Klein[3] states that on passing upward from the axis of an inflorescence into the pedicels of the individual flowers the following changes were observed:— (1) an increase in the amount of cortex, this increase being at the expense of the wood and pith, chiefly at the expense of

[1] The laboratory work for this paper was done under the direction of Prof. F. C. Newcombe in the botanical laboratory of the University of Michigan. The writer desires to thank Prof. Newcombe for his kindness and assistance, not only while under his immediate direction, but also while preparing the notes for publication.

[2] Oels, W., Bau des Stengels und der Blüthenstandaxe bei den Droseraceen. Inaug. Dissertation, Breslau, 1879. Ref. in Bot. Jahresbericht, 1879, 1. Abt., 43.

[3] Klein, O., Beiträge zur Anatomie der Inflorescenzen. Inaug. Dissertation, Berlin, 1886. Ref. in Bot. Jahresbericht, 1886, 1. Abt., 901, and Bot. Centralblatt, 1887, 32; 107–110.

the latter; (2) in the flower-stalk there was often no pith, because the bundles were crowded together in the centre. This became even more marked in the fruit-stalk, in which the necessity of an abundant supply of building-material and of mechanical support for the fruit resulted in the bundles taking a more central position.

Nanke[1] in 1886 worked on *Pirus Malus* and on *P. communis*, as well as on several other plants. He found that the cells in the fruit-stalk were smaller than those in the leafy shoot. This difference was most conspicuous in the wood-cells.

In 1887 Dennert[2] published the results of an examination of 180 species. He found an increasing delicacy of structure in passing from the leaf-shoot into the axis of inflorescence and thence into the pedicels. This change consisted in a weaker development of wood and pith from below upward. Dennert found that at the time of ripening of the fruit there was an increase in the development of mechanical tissue. This was manifested by an increase in the extent of xylem and in the thickness of the walls of the wood-fibres. The medullary rays of the stem were usually lacking in the fruit-stalk. An increase of hard bast did not occur where there was an abundance of wood, and vice versa.

In the same year Reiche[3] presented some work on additional species. His work confirms the results obtained by preceding authors.

In all this work the flower-stalk or the fruit-stalk was compared with the leafy stem. It was a study in the adaptation of organs to the demands made upon them, as

[1] Nanke, W., Vergleichend-anatomische Untersuchungen über den Bau von Blüthen und vegetativen Axen dicotyler Holzpflanzen. Inaug. Dissertation, Königsberg, 1886. Ref. in Bot. Jahresbericht, 1886, 1. Abt., 902, and Bot. Centralblatt, 1886, 32, 33; 145–147.

[2] Dennert, E., Metamorphose der Blüthenstandaxen: Bot. Hefte, Forschungen aus dem Bot. Garten zu Marburg, Heft 2, 128–217, 1887. Ref. in Bot. Jahresbericht, 1887, 2. Abt., 647.

[3] Reiche, K., Beiträge zur Anatomie der Inflorescenzen; Berichte der Deutschen Bot. Gesells., 1887, V, 310–318.

manifested in their histology. The same may be said of Laborie's work[1], although he used fruit-bearing and vegetative shoots, instead of comparing fruit-stalks with vegetative shoots. This brings him nearer to the purpose of this paper, although he kept in view the study of adaptation to use as the principal end of the work, and made a more detailed histological study than is here contemplated. He found a marked preponderance of parenchymatous tissue, both cortical and medullary, and a more feeble development of the fibrovascular bundles, in the fruit-bearing shoot. The primary bast was nearly always lacking in the fruit-bearing shoot, or if present it was thin and scattered. In this shoot also the crystal-bearing cells and sieve-tubes were more abundant; and in the xylem there were fewer fibres, their place being taken by wood-parenchyma.

The first paper I found in which the influence of fruit-bearing on the structure of the woody axis was considered was one by Sorauer[2], who, in 1880, published the results of an investigation on the effect of fruit-bearing on the development of wood in some fruit-trees. I regret that I have not had access to the original paper, but I have made use of two excellent abstracts, one in the Bot. Centralblatt for 1880, p. 453, and one prepared for me by Prof. F. C. Newcombe while at Leipzig. The latter paper has been especially helpful, since it gives the gist of Sorauer's thought in a few concise quotations.

Sorauer made comparative measurements of the diameters of internodes in shoots of wild and cultivated pear-trees, and also of fruit-bearing and leafy shoots on cultivated trees. From these measurements he concluded that cultivated varieties are weaker (*weicher*) than wild ones, and that fruit-bearing shoots are weaker than leafy ones; also, that in any

[1] Laborie, Note sur les variations anatomiques et la différentiation des rameaux dans quelques plantes, Compt. Rendus, 1883, 97; 342.

[2] Sorauer, P., Beiträge zur Kenntniss der Zweige unserer Obstbäume. Forschungen auf dem Gebiet der Agrikultur-Physik, 1880, Band III, Heft 2. Ref. in Bot. Centralblatt, 1880, 1; 453.

shoot the base is firmer than the apex. The interpretation of the term '*weicher*' to mean mechanically *weaker* as well as *softer* seems justified by a sentence from the abstract in the Bot. Centralblatt, as follows: 'Bei den Kulturvarietäten bildet der Holzring einen kleineren Theil des Dickendurchmessers eines Zweiges als bei den Wildlingen. "Kulturvarietäten sind weichholziger," sagt die Praxis.' The entire argument also tends to show that the thought in Sorauer's mind was that a shoot on a cultivated tree was less able to bear mechanical strain than one on a wild tree. He tabulated his results to show the different percentages of wood and of cortex in the shoots examined. These percentages are in terms of the pith. No reason for using the measure of the pith as a basis is given in the abstracts, but there is one objection to this method which I wish to point out. The apparent difference in the relative amounts of wood in the different shoots is much greater than it would be were the percentages given in terms of the diameter of the shoot. Sorauer's last table, in which he summarizes his results, will serve as an illustration:—

	Cortex.	Wood.	Pith[1].
Wild pear . .	75 %	80 %	100 %
Cultivated pear .	91·4 %	58·2 %	100 %

This shows a considerable difference in the percentages of wood and cortex; but, if the percentages are given in terms of the diameter, we have the following for the same shoots:—

	Cortex.	Wood.	Pith.
Wild pear . .	36·6	39·6 +	23 +
Cultivated pear .	45 +	29 +	26

The actual measurements from which all the above percentages were determined are as follows:—

	Cortex.	Wood.	Pith.	Wood.	Cortex.
Wild pear . .	24·8	27·9	33·3	25·4	25·2
Cultivated pear:					
Base of internode	58·7	42	67·3	42·7	72·3
Middle of internode	66·3	41	76·5	41·2	65·5

[1] The pith is not in the original, but it is put here for the sake of clearness.

When we use the diameter of the shoot as a basis, a truer conception is afforded of the difference between the two shoots; since the important question is not what relation the size of the wood-cylinder bears to that of the pith, but what relation it bears to the diameter of the shoot. It must be borne in mind, too, that, although the wood does not increase in the same proportion as does the cortex, yet there is a considerable increase in the diameter of the xylem-cylinder; and it still remains to be shown that it is necessary for the well-being of the tree that the proportion of increase shall be the same in all the tissues.

Sorauer thinks that the increase in cortex is due to the need for greater food-supply, and he states that the increased proportion of parenchymatous tissue brings with it a greater liability to injury by frost. Finally, he claims that absolute size has no value in a comparison of results. It is, he says, the proportionate amount of xylem that determines the strength of a shoot.

In a later paper Sorauer[1] reiterates his former conclusions. He also attributes to over-cultivation certain swellings on shoots, which he finds are due to a parenchymatous change in some of the wood-cells. I have not found any of these growths, and cannot therefore treat of them in this paper.

The inference drawn from Sorauer's work is that cultivation, and more directly fruit-bearing, may become injurious to a tree by reason of the greater development of cortex and the proportionally smaller amount of xylem produced in the fruit-bearing shoot, which renders it weaker mechanically and more liable to injury by frost.

I shall present here a study of the effect of fruit-bearing on the permanent mechanical[2] tissue of the tree, in order to

[1] Nachweis der Verweichlichung der Zweige unserer Obstbäume durch die Cultur. Zeitsch. f. Pflanzenkrankheiten, Bd. II, 1892, pp. 66–70 and 142–148.

[2] By 'mechanical tissue' is meant all those collections of cells having thick and lignified walls, and serving to give strength and firmness to the shoot. Since the wood-cylinder is the principal collection of such cells, and is the only one capable of accurate measurement, most attention will be given to it, with incidental reference to supplementary mechanical cells when these are of importance.

show the influence upon any tissue-system of the strain to which a tree is subjected in the production of fruit and seeds.

Material and Methods.

The trees studied are Apple, Pear, Peach, and Plum. The shoots to be compared were selected with reference to similarity in size and vigour, and were taken in each species from the same tree, often, indeed, from the same branch, in order to avoid, as far as possible, the influence of dissimilar conditions. When collected, these shoots were numbered and put into 50 per cent. alcohol, in which, to avoid unequal shrinkage, all were left for the same length of time.

To study the shoots, free-hand cross-sections were made, and the tissues measured with an eye-piece micrometer under a power of about 100 diameters. The measurements were made in two diameters at right angles to each other, and the average thickness of the zones of tissue determined[1]. For comparing fruit-bearing and vegetative shoots these figures were reduced to percentages, which are given in terms of the diameter of the shoot. The figures were carried to two decimal places, and the remainder, if less than 0·005, was ignored, and if greater was counted as 0·01 and added to the result. Further details concerning the material and methods used will be given when the different species are discussed.

Discussion of Species.

The Apple[2].—A study of the influence of fruit-bearing upon the mechanical tissue of a tree involves two points: (1) the immediate and temporary effect, and (2) the permanent effect.

[1] For illustration a sample measurement is given as follows:—

		Cortex.	Wood.	Pith.	Wood.	Cortex.
Section 2, pear	First diameter	70	40	135	45	70
	Second diameter	73	45	137	60	90
Average . . .		71·5	42·5	136	52·5	80

[2] The trees selected for study were of the Rhode Island Greening variety.

The first point can best be studied by a comparison of one-year-old fruit-bearing shoots and one-year-old vegetative shoots, while a study of the second point requires that such shoots shall be chosen as promise to become permanent parts of the tree. Shoots fulfilling these conditions are found at the ends of the branches. Much of the fruit on both the Apple and the Pear is borne on short lateral shoots, which persist for a term of years, but which never become a part of the body of the tree. These shoots are known as 'fruit-stubs.' Their structure and its relation to the question under consideration will not be discussed in this paper.

By a one-year-old fruit-bearing shoot is meant that part of the terminal growth made during the season of collecting, and which lies between the scars left by the preceding winter's bud-scales at one end, and the bases of the fruit-stalks at the other. When collected in the fall, these shoots have completed one year of growth from their formation in the buds the previous year. The vegetative shoots selected occupied the same relative position, and were of the same age as the fruit-bearing shoots.

When growth begins in spring, the axis of the flower-bud lengthens rapidly, and bears at its apex a cluster of blossoms. Its complete growth in length is soon reached, and its continued vegetative existence is assured by the production of one or more lateral buds, which may or may not grow out into shoots during the same season. The fruit-bearing shoots average about 1·5 cm. in length, and are always more or less swollen. The swelling begins at the basal zone of scars, and is greatest at the apex of the shoot, just below the scar left by the breaking away of the fruit-stalk. By this scar, as well as by the swelling, a portion of a branch that has borne fruit during its first year may be recognized even until twelve years old. The age of such a part may be readily determined by counting the zones of bud-scars left at the base of each year's growth.

The shoots, both fruit-bearing and vegetative, were studied from cross-sections made serially from the basal zone of scars.

For a proper comparison of tissue-masses it was important that the sections should be cut at points having the same relative position. The basal zone of scars offered such a point, therefore only sections taken from this point were used for comparative measurements.

The material was studied with reference to the following questions:—

(1) Is the xylem-cylinder in a one-year-old fruit-bearing shoot less well developed than in a vegetative shoot of the same age and apparent vigour, and does it form a smaller proportion of the diameter of the shoot?

(2) What influence does fruit-bearing exert upon the lignification of cell-walls?

(3) Does the fruit-bearing shoot contain any supplementary mechanical tissue formed to supply a possible lack of development in the xylem-cylinder?

Although a detailed histological study of the swollen part of the fruit-bearing shoot is not contemplated, a general sketch of the arrangement of tissue, as found in a shoot gathered during October, may properly precede the study of tissue-masses.

At the base of the year's growth the structure is fairly uniform on all sides. The cortex is compact, the xylem-cylinder is dense, and the walls of the wood-cells are well lignified. A little above the basal zone of scars, the cortex begins to enlarge and intercellular spaces become common. At this point also the wood-cells show thinner walls, though the thickness of the xylem-zone increases somewhat. Above the middle of the shoot, the symmetry of the wood-zone becomes disturbed to a marked degree. The bulk of the xylem is on the side of the lateral vegetative bud, and here also the wood-cells have thicker walls than those on the opposite side. Nearer the apex the walls of the wood-cells on the side nearest the fruit-stalk are still thinner, often indeed so thin that it becomes impossible to determine the exact position of the cambium. The cortex, on the other hand, greatly increases in size. The cells are larger, the intercellular

spaces more frequent, and the supply of crystals and of starch is abundant.

In the upper part of the shoot the parenchymatous character of the cells increases until, on the side nearest the fruit-stalk, very little wood is present, and such secondary wood-cells as are formed are not lignified. Above the lateral vegetative bud there is no cambium, and no secondary wood appears; but here the supplementary mechanical cells, bast and sclerenchyma, become prominent. Groups of sclerenchyma-cells appear in the cortex and in the medullary rays; the pith is usually lignified; hard bast is present in great quantity and continues, but decreases in amount down to the base of the shoot, where it disappears.

The presence and arrangement of the hard bast is interesting. In the fruit-bearing shoot it appears in great abundance, its distribution being the reverse of that of the wood, namely, the greatest amount at the apex, where there is least wood, and decreasing until there is none at the base, where the wood is well developed. In vegetative one-year-old shoots of Rhode Island Greening, I found no bast-fibres. In the mature fruit-stalk the greater part of the cortex as well as the pith becomes lignified.

It is clear that the upper portion of the fruit-bearing shoot has a weak development of xylem. The wood-cells decrease in number and in the thickness of their walls until the xylem disappears entirely. This is, however, the case only in the upper portion of the shoot, which subsequently dries up and falls away. But this weakness is more than compensated for by the abundant supply of well lignified sclerenchyma and hard bast-cells. These are found in groups and bundles where the xylem is weak, decreasing where the latter becomes strong.

The comparative development of the xylem-cylinder at the bases of the fruit-bearing and vegetative shoots had, of course, to be determined by measurements of the tissue-zones in the cross-sections of the two kinds of shoots. The results of these measurements are embodied in the tables given subsequently.

The first table shows the percentage of each tissue and the amount of difference in the development of the tissues in the shoots. In the second table are given the actual measurements of the tissue-zones. These furnished the data from which the percentages in the first table were derived.

TABLE 1.—*Average percentage of tissue in one-year-old shoots of Rhode Island Greening Apple.*

	Cortex. Per cent.	Wood. Per cent.	Pith. Per cent.
Twelve vegetative shoots	40·74	21·48	37·77
Twelve fruit-bearing shoots	47·97	19·36	32·66
	+7·23	−2·12	−5·11

The percentages given in the above table were computed from the figures in the following table:—

TABLE 2.—*Average absolute amount of tissue in*[1]—

	Cortex.	Wood.	Pith.	Total.
Twelve vegetative shoots	90·6	47·77	84	222·37
Twelve fruit-bearing shoots	102·8	41·50	70	214·30
	+12·2	−6·27	−14	−8·07

Both the above tables show a larger amount of cortex in the fruit-bearing than in the vegetative shoot, while the wood and pith are more abundant in the latter. The difference in the amount of wood is small, reaching little more than 2 per cent., or only 94·05 micromillimetres in absolute measure. At the base the fruit-bearing shoot is smaller than the vegetative shoot. The characteristic swelling begins a little above the basal zones of scars. Above this point both the cortex and the wood increase in size, but the proportion of cortex increases most rapidly.

To determine the relative thickness of the walls of the

[1] In terms of the spaces of the eye-piece micrometer. Each space equals 15 micromillimetres.

wood-cells in the fruit-bearing and in the vegetative shoots, sections were taken near the bases of both; camera drawings were made of the wood-cells in a certain area; and the paper covered by the drawing was weighed. The portions representing cell-cavities were then cut out and the remainder again weighed. It was found that the weight of the paper representing cell-wall was 63·64 per cent. of the weight of the entire section in the case of the vegetative shoot, and 46·72 per cent. in the fruit-bearing shoot. This difference in the thickness of the walls became greater near the apex of the shoot, where many of the cells remain thin-walled until the following year, when their walls become thicker and are lignified. The lignification of the wood-cells proceeds from below upward, and from the younger secondary cells inward to the older primary ones. The primary vessels themselves are always first lignified, but the cells surrounding them and the secondary cells, formed early in the season, remain soft-walled until after the more lately formed secondary cells have become lignified.

The figures given above as representing the relative thickness of the walls of the wood-cells in the two kinds of shoots must not be regarded as average ones, since only one test was made. But there is no doubt that, as a rule, the wood-cells in the one-year-old fruit-bearing shoot have thinner walls than the cells in vegetative shoots of the same age.

The young fruit-bearing shoot must support the weight of the apple, but ample provision has been made for this in the abundant supply of hard bast and sclerenchyma. In view of this splendid development of supplementary mechanical tissue, the slightly smaller proportion of xylem in the fruit-bearing shoot cannot be considered a serious weakness for the first year. But it remains to be seen whether the relations existing at that time continue during the life of the shoot. To answer this, small branches were gathered, portions of which, now three and five years old, showed by their swellings and fruit-scars that they had borne fruit during their first year's growth. Other portions of branches were gathered, of

the same age as the first, but upon which fruit had never been borne [1].

A study of these shoots showed a remarkable development in the fruit-bearing ones, both in size of the xylem-cylinder and in the thickness of the walls of the wood-cells. Most of this increase had been on the side nearest the fruit-scar, thus making the radii of the xylem-cylinder more nearly equal. The walls of the wood-cells were apparently as thick and as well lignified in the fruit-bearing shoots as in the vegetative shoots. Measurements of the tissues showed that the wood-cylinder in the fruit-bearing shoot had not only outgrown its former weakness, but that it now formed a greater proportion of the diameter of the cross-section than was the case in the vegetative shoot.

TABLE 3.—*Amount of wood in the fruit-bearing and vegetative shoots at the end of the first, third, and fifth year of growth.*

Twelve vegetative shoots, first year	47·77
Twelve fruit-bearing shoots ,, ...	41·50
	−6·27
Seven vegetative shoots, third year	123·5
Seven fruit-bearing shoots ,, ...	133·8
	+10·3
Six vegetative shoots, fifth year	164·66
Six fruit-bearing shoots ,, ...	188·33
	+23·67

This increase in the development of the xylem, as well as in the walls of the wood-cells, indicates that the weakening effect of fruit-bearing upon the wood is temporary, and is quickly outgrown. That even the temporary effect is compensated for by the great development of supplementary mechanical tissue is shown above.

Pear.—The fruit-bearing one-year-old Pear-shoot resembles

[1] To avoid using a cumbersome descriptive phrase, these three- and five-year-old portions of branches will hereafter be designated as three- and five-year-old fruit-bearing and vegetative shoots respectively.

that of the Apple in every way, except size. It is larger, and the swelling is greatest near the middle, decreasing from this point in both directions. The upper part withers after fruit-bearing. In arrangement and division of tissue the part below the insertion of the lateral vegetative bud is similar to the corresponding part of the Apple. Instead, however, of bast-fibres, the supplementary mechanical tissue is sclerenchyma. The sclerenchyma-cells are slightly elongated and taper-pointed, with thick and strongly lignified walls. Bundles of these cells are placed just outside of the fibro-vascular bundles, and are scattered throughout the cortex, medullary rays, and pith in the upper part of the shoot. Like the bast-fibres in the Apple, they decrease in number near the base of the shoot.

An early variety of Pear, of unknown name, was selected for study. Measurements were made in the same manner as for the apple, the sections being taken near the base in each case. Although the largest possible vegetative shoots were used, they were, as a rule, no longer than the fruit-bearing ones.

TABLE 4.—*Average percentages of tissue in one-year-old shoots.*

	Cortex.	Wood.	Pith.
Five vegetative shoots .	42·20	23·12	34·68
Five fruit-bearing shoots .	41·36	22·10	36·54
	−0·84	−1·02	+1·86

There is very little difference in the proportionate amount of tissue in these shoots, but an examination of the tables of absolute measure will show that the advantage, as far as amount of mechanical tissue is concerned, is largely with the fruit-bearing shoot.

TABLE 5.—*Average absolute measure of tissues in the same shoots as in Table* 4.

	Cortex.	Wood.	Pith.	Total.
Vegetative shoots .	106·6	58·4	87·6	252·6
Fruit-bearing shoots .	146·0	78·0	129·0	353·6
	+39·4	+19·6	+41·4	

The diameter of the fruit-bearing shoots at the base is much greater than that of the vegetative shoots. This is largely because the swelling begins at the base, involving the basal zone of scars. It is impossible to get entirely below it and still be within the year's growth.

The tables show that the amount of woody tissue is greater in the fruit-bearing than in the vegetative shoots, although the percentage is slightly less. This is due primarily to the increased size of the pith, since the proportion of cortex is also greatest in the vegetative shoots. The cortex, however, increases rapidly in size toward the middle of the shoot, and soon greatly exceeds that in the vegetative shoot. The walls of the wood-cells in the fruit-bearing shoot at the base were of usual thickness, as far as could be ascertained by careful inspection. No measurements were made.

It is clear that the base of the fruit-bearing shoot is not mechanically weak.

Plum.—The material for this work was collected in the latter part of July. Two sets of shoots were taken, both in their third year of growth. One set bore mature fruit at the time of collecting, and the other had borne no fruit that season.

In the Plum, as in the Peach, the axis of the fruit-bud is but slightly developed, and the fruit-stalk seems to proceed directly from the branch upon which the bud is borne. The sections of the Plum were taken 1 or 2 centimetres below the point of attachment of the fruit, and, in the vegetative shoot, at a point as nearly similar to this as possible. The tissue from ½ to 1 centimetre below the fruit shows the effect of fruit-bearing. The zone of cortex is larger than either above or below this point; the zone of xylem and the pith are also larger. The development of all the tissues is greatest on the side toward the fruit, making the section of the stem at this point oval instead of circular. The walls of these wood-cells are thin and but little lignified. The measurements were taken from sections cut below the swollen portion in order that those from fruit-bearing and from vegetative shoots might be comparable.

The Plum-shoots bear fruit during their second and third years. The scars left by the fruit-stalks are so small that their presence cannot be certainly determined. It is therefore not known whether any of the three-year-old shoots used bore the previous year or not.

By the term vegetative shoots is meant those not bearing fruit during the season of collecting, and by fruit-bearing shoots those of the same age bearing mature fruit at the time of collecting.

TABLE 6.—*Average proportions of tissue in*—

	Cortex.	Wood.	Pith.
Five vegetative shoots three years old	26·6	50·8	22·6
Five fruit-bearing shoots „ „	30·79	44·77	24·44
	+4·19	−6·03	+1·84

The figures given in this and the following tables on the plum may not be entirely reliable, because the history of the shoots used is unknown. If the vegetative shoots of 1894 bore fruit during 1893, the apparent influence of fruit-bearing would be decreased. It would show that the effect of previous fruit-bearing did not prevent the shoot from developing a greater amount of wood than was formed in the fruit-bearing shoot of 1894. In this case the difference in the amounts of tissue would have to be attributed to unknown accidental causes. If, on the other hand, the vegetative shoot had not borne fruit the previous year, it would tend to verify the supposition that all the difference between the shoots was due to fruit-bearing. The figures in Table 6 were computed from the data given in the following table:—

TABLE 7.—*Average absolute amount of tissue in*—

	Cortex.	Wood.	Pith.
Five vegetative shoots . . .	65·4	125	55·6
Five fruit-bearing shoots . . .	73·6	107	58·4
	+8·2	−18	+2·8

A greater difference between the two kinds of shoots in the amount of woody and parenchymatous tissue is found in

the Plum than was found in the Apple or Pear. However, satisfactory results could only be obtained by taking sections of shoots whose fruit-bearing history was known for several years past, and measuring the amount of wood formed each year. A comparison of the amount formed during fruitful and unfruitful years would show the effect of fruit-bearing and whether any such effect was constant.

A few trees of the Wild Goose variety of Plum were found whose history for the past three years was known. During 1891 the trees bore a heavy crop of fruit, but bore no fruit at all during 1892 and 1893. In the spring of 1894 shoots were cut from different parts of one of these trees for the purpose of studying the effect of a heavy crop upon the amount of wood formed during the fruit-bearing year, but with no special reference to the shoot upon which the fruit was borne.

In the following table the measurements are given, as before, in terms of the eye-piece micrometer, each having a value of 15 micromillimetres[1].

TABLE 8.—*Average amount of wood formed during*

	1893.	1892.	1891.	1890.	1889.	Aver. of
1 year old .	62					4
2 years old .	54	37				5
3 years old .	35	52	45			6
4 years old .	30	34	34	42		4
5 years old .	25	43	30	35	29	2
	No fruit borne.	No fruit borne.	Fruit-bearing year.	Unknown.	Unknown.	

The average amount of wood formed in the shoots three, four, and five years old, during 1891, the fruit-bearing year, is 36; during 1892, 43; and during 1893, 30. The average annual amount formed in these shoots during these three

[1] It should be borne in mind that throughout the paper all actual measurements are given in units of the eye-piece micrometer, each unit being equal to 15 micromillimetres.

is $36\frac{1}{8}$, only a trifle more than the average of all of them for 1891. If fruit-bearing was the principal factor in reducing the amount of wood, we should expect its effects to appear more strongly in 1892 than in 1893; but more wood was formed in both 1891 and 1892 than in 1893.

Peach.—The material for the study of the Peach was collected while the ripe fruit was still on the tree. Some of the shoots used bore two peaches, so near together as to be almost opposite. The vegetative shoots were selected as nearly like the fruit-bearing ones as possible, and of course of the same age. Sections near the base of the fruit-stub, as well as from points above and below the fruit, were studied. Measurements, with one exception, were made from sections cut about 2 centimetres below the attachment of the fruit. This avoided the local effect of fruit-bearing.

The peach is borne on the shoot making its second season's growth. It is supported by a rigid stub 2 or 3 millimetres in length. This stub is remarkable for the great amount of mechanical tissue it possesses. The wood-cylinder is dense, the walls of the wood-fibres are thick and well lignified.

The modifications described above for the Plum also occur in the Peach-shoot. Just below the stub the stem is swollen and the tissues have the greatest development on the side toward the fruit. This irregularity is local; at a distance of 1 to 2 centimetres it has entirely disappeared.

Great care was taken to cut the sections for measuring the tissues from points as closely comparable as possible and to have the shoots alike in size and vigour. All the shoots used were two years old. The tables which follow show that, in the Peach, fruit-bearing certainly does not produce a weak development of xylem. The effect is local and is confined to a very limited area. The proportion of xylem in the fruit-bearing shoot is greater than that in the vegetative, while the proportion of cortex is greater in the latter. The cortex in the fruit-bearing shoot of both the Plum and Peach is not as well developed as in the corresponding shoots of the Apple and Pear; the proportion of pith is smaller in the

fruit-bearing than in the vegetative shoot, and relatively smaller in the swollen portion than elsewhere.

TABLE 9.—*Average proportion of tissues in—*

	Per cent.		
Five vegetative shoots . .	35·22	33·98	28·78
Five fruit-bearing shoots .	30·91	39·69	19·25
	−4·31	+5·71	−9·53

In the small swollen area at the base of the fruit-stub the zone of wood is thicker than it is a little lower in the same shoot, but the walls of the wood-cells are thin and mostly unlignified.

TABLE 10.—*Actual average measurements of the shoots and measurements through the swollen part.*

	Cortex.	Wood.	Pith.	Total.
Five vegetative shoots .	65	63	53	181
Five fruit-bearing shoots .	59	77	55	191
Five fruit-bearing shoots through swollen part .	78	87	53	218

SUMMARY.

The study of these four species seems to warrant the following conclusions, in answer to the questions proposed at the beginning of this paper:—

1. The one-year-old fruit-bearing shoots of the Apple and the Pear have less wood in proportion to their diameter than does the vegetative shoot of the same age. This is due, in the Apple, largely to an increase in the cortex, and in the Pear solely to a great increase in the cortex and the pith, of the fruit-bearing shoot. It does not, however, appear from the structure of the shoots that the fruit-bearing shoot is weaker than the vegetative. The former is well supplied with supplementary mechanical tissue, which is distributed at those points where it is most needed, and thus gives it an increase of strength for the fruit-bearing year which fully makes up for the difference in xylem-development.

2. In the Peach the fruit-bearing shoot has more wood than the vegetative shoot, and the walls of the wood-cells are as thick in the former as in the latter.

3. In general it may be said that the effect of fruit-bearing upon the tissues is local. In the Apple and Pear it is perceptible throughout the one-year-old shoot; in the Plum and Peach it is confined to a small area in the immediate neighbourhood of the fruit-stalk.

4. The local effect of fruit-bearing tends to an increase of cells, with a decrease in the thickness and lignification of the walls of the wood-cells. The cortex is especially enlarged, giving rise in the Apple and Pear to the swollen condition of the fruit-bearing shoot.

5. In all cases the increase in growth is greatest on the side near the fruit-stalk, although the wood in the Apple and Pear is best developed on the side of the lateral vegetative bud.

6. The local effect of fruit-bearing on the wood-cylinder disappears with time. The study of Apple-shoots that had borne fruit during their first year showed that in the two or four years following there had been a rapid increase of wood, especially on the side of the fruit-scar. This side was weakest at the end of the first year. These shoots at the end of three and five years had a better xylem-development than shoots of the same age that had never borne fruit.

7. Fruit-bearing has a temporary local effect upon the lignification of the walls of wood-cells. It prevents their lignification wholly or in part, according to their distance from the fruit-stalk. The lignification of other cell-walls is promoted by fruit-bearing. In the fruit-stalk the greatest part of the tissue has become lignified, and in the upper part of the Apple- and Pear-shoot there is an abundance of well-lignified sclerenchyma and hard bast, which is either not found in the vegetative shoot or only sparingly so.

The Respiration of Wounded Plants.

BY

HERBERT MAULE RICHARDS.

With Woodcuts 2 and 3.

WHILE it has been known for some time that, when wounded, plants respire with greater activity than under normal conditions, a more precise knowledge of the phenomena connected with this increased respiration and the causes thereof has been lacking. Böhm [1] called attention to a considerable rise in the amount of CO_2 produced by potatoes which had been injured by cutting in various ways; but the question was not further investigated until Stich [2], in connexion with other researches, published a more extended account of this phenomenon in the same and other plants. He established the fact of the increased respiration more clearly than Böhm had done; but, as the period of his experiments was short, he did not determine the ultimate effect of the injury on the CO_2-production as compared with normal conditions. One of the most interesting facts recorded is that when the cut surfaces of the potatoes were so fixed together with neutral gelatin as to exclude the access of air the amount of respiration in excess of the normal was markedly less. More remains to be said concerning this point, and it will be taken up later, after a discussion of experiments made by the

[1] Botanische Zeitung, Vol. xlv, p. 671, 1887.

[2] Die Athmung der Pflanzen bei verminderter Sauerstoffspannung und bei Verletzungen, Flora, Vol. xlix, p. 1, 1891.

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writer. Since his first paper[1] Böhm has published a short note, in which he gives, as the most probable explanation of the increased respiration, a traumatic action of the wound itself not depending upon the action of the atmospheric oxygen on the tissue exposed by wounding. To arrive, if possible, at some definite conclusion regarding these points was the object of this research. The work was undertaken at the suggestion and under the direction of Prof. Pfeffer, to whom the writer's best thanks are due for his advice, and for the opportunities offered for carrying on the research.

After a preamble concerning the methods employed and the apparatus used, there is a discussion of the various experiments, after which follows a record of the experiments in tabular form. The latter are referred to in the text by number or letter.

Apparatus and Methods.

By far the larger amount of the work was done with the Pfeffer-Pettenkofer apparatus for determining the respiration of plants, and with a modified form of Stich's apparatus for finding the $\frac{CO_2}{O_2}$-equation. A few experiments were also made to obtain some idea of the amount of CO_2 contained in living tissues, which will be mentioned in passing.

A Pettenkofer apparatus of the form as improved by Pfeffer[2] was employed; the apparatus is so well known that it is unnecessary to dwell on it, and in the reference given a full description of it will be found. It is also scarcely necessary to rehearse the sources of error to be avoided and the various precautions which must be taken to ensure accuracy, as all of these have been described with care by earlier writers. It suffices to say that every care was taken to observe the necessary precautions; that the apparatus was tested from time to time to make sure that there were no leaks; that the KOH

[1] Bot. Centralblatt, Vol. l, p. 200, 1892.

[2] Ueber intramolekulare Athmung: Untersuch. aus dem Bot. Inst. zu Tübingen, I. 636.

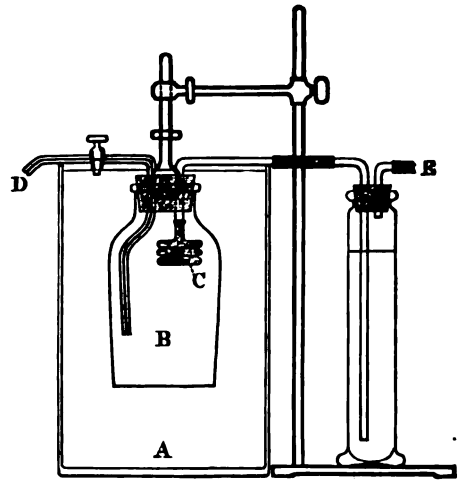
in the U-tubes for washing the air from CO_2 was frequently renewed; that care was taken that the air supplied to the plants in the receiver should be sufficiently moist, and so forth. All the manipulations of the experiments and analyses were carried on each time in as nearly a similar manner as possible to ensure equality in the results. For the analyses the same burette and pipettes were used throughout the whole course of the work. The titrations were made with a solution of hydrochloric acid of such a strength that 1 cc. equalled 1 mg. of CO_2, and for an indicator phenol-phthalein was employed.

The constant flow of air or gas through the apparatus was obtained by means of a Stainer's drop-aspirator regulated with a gas-meter (Experimentir-Gas-Messer of Elster), by which means no difficulty was found in keeping the current within 50 cc. per hour of the amount desired. The rapidity of the current was nearly 3·5 litres per hour, an amount to which the apparatus held with surprising constancy throughout the time it was used. When gases other than air were run through the apparatus, it was necessary to be somewhat careful to regulate the pressure from the gasometer so as to avoid an increase in the gas-stream. Even in such cases, however, no difficulty was found in keeping the flow at the desired rate. The hydrogen and oxygen used were first washed roughly at the time of preparation with $KMnO_4$ and KOH, and again, when being used, by means of U-tubes containing $KMnO_4$, KOH, and $HgCl_2$ interpolated between the gasometer and the receiver containing the plants.

In every case the receiver containing the plants was immersed in a large vessel of water, and thereby kept at an approximately constant temperature, which usually varied but little more than half a degree during the day, the average temperature being about 20·5°.

For the determination of the relative amounts of CO_2 produced and of O_2 absorbed, or, as it is usually expressed, the equation $\frac{CO_2}{O_2}$, a form of apparatus somewhat different from

both that of Godlewski [1] and of Stich [2] was employed. In this case the O_2 absorbed was not measured by the diminished gas-volume in the receiver, but samples of the air in it were taken and analyzed directly for the CO_2 and O_2 which they contained. The apparatus was exceedingly simple, and thereby the sources of error arising from its use the more



WOODCUT 2.

Diagram of $\frac{CO_2}{O_2}$ apparatus.

A, Large vessel filled with water. *B*, Receiver for plants. *C*, Rubber-balloon. *D*, Capillary-tube leading to mercury bath. *E*, Tube leading to air-pump. Rubber indicated by shading; iron by heavy lines; all else glass.

easily eliminated. A bottle of some 750 cc. capacity was selected, the neck of which was not shallow, and preferably ground, and to this a deep stopper of soft rubber, perforated with two holes, was fitted. Through one of the holes was passed a capillary barometer-tube, one end of which reached almost to the bottom of the vessel, the other end leading off under a mercury-bath, being closed, however, near to the

[1] Pring's Jahrb., Vol. xiii, p. 491, 1882. [2] l. c., p. 7.

bottle by means of a cock. Through the other hole in the stopper was passed a piece of ordinary tubing, which projected a short distance down into the bottle. To this end was attached a rubber-balloon with very stout walls capable of considerable expansion; while the other end, bent at a short distance above the stopper, led off to a bottle filled with water, to which was attached an air-pump. When a few strokes were given to the air-pump, the water in the bottle referred to was forced into the balloon, thus expanding it to three or four times its original capacity, by which an appreciable pressure was produced in the receiver. Reference to the figure (woodcut 2) will explain the details of this apparatus. The whole receiver was immersed under water until the stopper was well covered, to ensure both perfect closing and a tolerably constant temperature. As the temperature of the laboratory was fairly regular, it was possible to keep the receiver within at most one degree of the same temperature with comparative ease.

The mode of operating was as follows. The samples of plants under investigation were put into the receiver, and after a current of air had been passed through it the stopper was fixed tightly in place and the whole clamped in position under the water. The cock leading to the delivery-tube was closed, and the glass tube from the rubber-balloon connected with the air-pump. Two or three strokes of the air-pump were given, and the balloon so expanded was allowed to collapse to drive out the air which it contained. The apparatus was then allowed to stand until the time for the experiment had expired, upon which a sample of the contained air was taken as follows. The balloon was first filled and emptied three or four times to procure a thorough admixture of the gases in the receiver; this was found by actual experiment with smoke to be thoroughly accomplished in three repeated fillings and emptyings. Finally the balloon was once more distended, the end of the capillary-tube thrust under the mercury, and the cock at once opened. A very considerable stream of gas was thus forced out, of which the first few cc. were rejected to

make quite sure of a pure sample of the gas in the receiver. The samples of gas for analysis were collected in little tubes over dry mercury. After each experiment the air was renewed, either by running a strong current of air through the receiver, or by removing the plants from the bottle and filling it with water.

As a control for the method, experiments were made with samples of air containing a known quantity of CO_2. This was analyzed at the beginning of the experiment, and again two days later, after the apparatus had been under water as described. As will be seen below in the three analyses given, the percentage of CO_2 does not vary appreciably between the beginning and end of the experiments :—

No. of experiment	I.	II.	III.
% CO_2 at beginning . . .	5·94	11·35	8·42
% CO_2 48 hours afterwards . .	5·92	11·37	8·38

The danger of leakage as a source of error having been shown to play no part, the only other errors to be considered are those possibly arising from change of temperature during the course of the experiment, or from the danger of the air in the capillary delivery-tube vitiating the sample taken ; but these were guarded against in the manner described. While the plants under experiment were plentifully supplied with moisture, care was taken to allow no considerable amount of water to remain in the receiver, as a source of error due to the absorption of CO_2 thereby might arise. The agreement of the results among themselves speaks for the accuracy of the methods employed.

For the analysis of the air one of the improved Bonnier and Magnin apparatuses was used, such as is described by Aubert[1]. By observing all the precautions against errors described by the above writers, it is possible to obtain results of more than sufficient accuracy for this work, and a similarity of manipulation tends to equalize any other remaining sources of error. Repeated analyses of the air in the laboratory showed that, as far as the purposes of this research are concerned, the amount of CO_2 contained in it need not be

[1] Rev. Gén. de Botanique, Vol. iii, p. 97, 1891.

regarded, being a scarcely perceptible fraction of one per cent. In all cases the percentage of O_2 in air was taken as 20·8, being the average amount shown by repeated analyses with this apparatus, and agreeing well enough with the already known percentage.

For certain determinations of the amount of CO_2 contained in thick bulky tissues such as potatoes, carrots, &c., a method was employed for driving the gas off with boiling water. A large flask, in which the potatoes or carrots were placed along with a considerable amount of water, was connected by means of a Liebig-condenser in reversed position with the Pettenkofer apparatus. The water was brought to the boiling-point as rapidly as possible, care being taken that no gas could escape from the flask, except through the tubes containing the BaO_2H_2, and also that no water passed over from the condenser into the tubes. The CO_2 evolved was estimated in the usual way by titrating the remaining BaO_2H_2. Almost all of the CO_2 thus driven off came over in the first ten minutes after boiling. The method is open to the objection that the high temperature required might bring about the decomposition of organic substances in the tissue, and thus give an excess of CO_2; but the fact that the gas comes off so suddenly would seem to point to its being merely absorbed or enclosed. The results were only accepted as approximate at best, and, as will be seen, no very great stress is laid on them.

All the material experimented with, with the exception of the leaves from the green-houses, was brought into the laboratory at least four days before using, to allow it to become accustomed to the temperature of the room. In almost all cases the respiration of the uninjured condition was first ascertained, the only exceptions being a few cases with potatoes, in which it was known that the respiration was practically none from experiments made previously with similar potatoes. The character of the plants and tissues employed for experimentation was various: bulky tissues like potato-tubers (*Solanum tuberosum*), carrots (*Daucus Carota*), &c.; thin, well-aerated tissues such as various leaves;

young growing tissues as found in seedlings (*Vicia Faba*, *Cucurbita Pepo*); and lastly, as with *Salix* twigs, tissue of an entirely different kind. The large majority of the experiments, however, were performed with potatoes, which lend themselves more readily to manipulation, and which react, as far as respiration goes, more markedly to injury than any of the other tissues tried.

The injury to which the plants were subjected consisted of cutting in various ways. Potatoes and carrots were usually cut into four with a sharp knife; leaves were slit longitudinally; while with seedlings the hypocotyl was split, or the tip of the root cut off. Immediately after injury, the tissues were always washed and only somewhat dried, both to rid them so far as possible from the cells killed in the process of cutting, and to ensure entire turgidity of the neighbouring parts. As Stich [1] has pointed out, the amount of CO_2 contained in the small quantity of liquid exuded from the cut surfaces is even in the case of potatoes practically imperceptible. In those experiments where it was desired to protect the wounded parts, Stich's method of sealing the pieces together with neutral gelatin was necessarily avoided, as the gelatin affords too good a basis for the growth of Bacteria when experiments lasting several days are to be undertaken. For this purpose clay was substituted. The clay employed was tested and found to be quite neutral. It was applied to the cut surfaces, which were then stuck together in their original position so that the oxygen of the air had no opportunity to act on them. After one day it was possible to remove the clay in large pieces without injury to the tissue beneath, after which the wound was washed in a stream of water, which, as shown below, does not cause any new increase in the amount of CO_2 produced.

	I. mg. CO_2 per hour.	II. mg. CO_2 per hour.
Potato three days after injury . . .	6·30	7·45
Same after washing and drying . . .	6·15	7·50

[1] l. c., p. 15.

Care must be taken, however, or portions of the corky layer which heals the wound will come off, and the fresh surfaces thus laid bare cause an increase in respiration.

During the night-time, or other times when determinations were not being made, the receivers containing the plants were placed in an inverted position to allow for the escape of the CO_2 formed; or, as was more often the case, connected with the aspirator, so that no abnormal conditions could occur owing to the lack of O_2 or the accumulation of CO_2 in the receivers. Throughout the course of the experiments the plants used were kept well supplied with moisture to keep them turgid, but no excess of water was allowed to stand in the receiver. A careful watch was kept for the growth of Fungi or Bacteria on the plants, but no difficulty was experienced from this cause, even in the experiments which were continued for a week or more.

Discussion of Results.

Concerning the ordinary course of the respiratory activity of an injured plant, it may be said in general that there is an increase, varying very much in point of time and extent with the character of the plant and the conditions to which it is subjected, followed by a subsequent decrease to the normal or almost normal respiration as the wound heals. Reference to any of the experiments will afford evidence of this; for it will be seen that an increase of more than 100 per cent. was often found during the period of maximum respiration. In the case of potatoes, carrots, and other bulky tissues, it will be observed that in almost every case there is, during the first two hours, a very sudden and marked increase, which, however, falls again rapidly, although to a point still considerably above the normal respiration, to rise more slowly later to the maximum production of CO_2. The causes of the first sudden increase will be discussed later; at present the general effect of the injury demands attention. The respiratory activity in these plants is at its height on the beginning of the second day, or

more precisely from twenty-four to thirty hours, after the injury has been inflicted, provided that the healing of the wound has been in no way retarded otherwise than by keeping the plants in a moist atmosphere. The course of this curve is fairly constant, and a decrease to a nearly normal rate of respiration, although much more gradual than the increase, is to be expected in from seven to eight days. In the case of the potatoes experimented with, the respiration never reaches quite so low a point as the normal, but this amount is in the first place so exceedingly small that hardly anything else could be expected when taking into consideration the increase of temperature and moisture to which the specimens were subjected. The amount of CO_2 evolved in comparison to the normal production is somewhat variable, but it will be noticed that it usually corresponds to about twice the amount found at the time when the plant is at its lowest respiratory condition after injury.

In potatoes the percentage increase of the respiration of the injured over the uninjured condition is far greater than in any other of the plants examined. It was with potato-tubers that Boehm[1] first observed the phenomena attending injury, and he also records a maximum on the second day, but in one instance only. Stich[2] in two cases gives a maximum at a somewhat earlier period, but that is a point which might readily vary with the conditions of the experiment. With carrots the maximum period (Expts. 14 and 15) was found not to be reached in some cases until the beginning of the third day. The first strong evolution of CO_2 during the first few hours is very well marked in carrots (Expts. 14 and 15), and is almost as great as the maximum reached later. In the same way sugar-beets and the ordinary red beet showed a similar respiration-curve after injury, although, weight for weight, the evolution of CO_2 is by no means so great as with carrots. As regards the $\frac{CO_2}{O_2}$-equation, it will be seen in general that, although between the uninjured and

[1] Bot. Zeit., Bd. xlv, p. 671, 1887. [2] l. c., p. 53, Versuche, 11 and 12.

injured conditions there is an appreciable rise in the fraction, there is no other marked change in the relations except during the first few hours after injury. The absorption of O_2 is always greater than the production of CO_2, except at the time already referred to, when the latter gas almost equals or slightly exceeds the former in quantity (Expts. 37, 41). There is a slight tendency to an increase of the CO_2 in relation to the O_2 at the time of the maximum respiration, which, however, falls off again somewhat as the respiration decreases.

In the experiments with more active tissues, for which seedlings of *Vicia Faba* were taken, results of a less constant nature were obtained. But in most cases a distinct increase in the respiration was found, rising to a maximum at a somewhat variable time after injury, and falling again to approximately the original amount as before injury. Necessarily, owing to the changes taking place in the seedlings during the course of the experiment, the reaction to injury was at times dwarfed by other causes. Stich, who experimented with a considerable range of seedlings, found a great diversity in the amount of respiration, but usually he records a distinct increase after wounding. One fact to be noted in the experiments with seedlings is that no sudden evolution of CO_2 is to be found directly after injury. This is also true of leaves, which show, however, a rise and fall in the respiration, as do other injured plants, although, necessarily from their short life after separation from the plants on which they grow, it is not for long.

Such, then, are the general facts connected with the phenomena of the respiration, which raise a number of questions that require a more detailed discussion of some of the experiments to answer. The first question is of course what, on the whole, is the cause of the increased respiration. As has already been said, Böhm[1] concludes there is no doubt that the cause of it is alone the irritation of the wound itself, and not the increased opportunity for the action of the oxygen of

[1] Bot. Centralblatt, Bd. 1, p. 200.

the air. He cites the results of some experiments with potatoes which had been hollowed out in the centre, the cavities being filled with water, and again sealed from the air, under which conditions their respiration still continued very strong. But on the other hand must be considered Stich's[1] results already referred to, which point to another explanation. Experiments similar to those of Stich's with gelatin were tried, but of longer duration, so that it was necessary to use clay for the covering of the wounded surfaces. In Experiment 7, with potatoes the quarters of which had been covered with clay at once after cutting, the four determinations taken on the day of the injury did not show the expected increase. On the next day, when the ordinary maximum for a similar quantity of potatoes should have been about 15 mg., it was only 7·60 mg.; while after the lapse of seven days the amount was practically nothing. After the removal of the clay there was a sudden and marked increase of the CO_2-production. The cut surfaces of the potatoes which had been protected by the clay were not absolutely fresh, being covered with a thin layer of corky material, which, by the way it may be mentioned, was not disturbed in the removal of the covering. This is shown even more clearly in the case of Experiment 13, where the clay was not applied until the time of the maximum respiration. Immediately there followed a decrease, which again gave place to an increase when about twenty-four hours afterwards the clay was removed. The carrots (Expts. 16 and 17) treated in a similar manner exhibited much the same effect; in both the cases cited the application of the clay hindered the usual increase of CO_2, and, when after some time the clay was taken off, the respiration at once rose to a very considerably higher point, where it remained, with only a slight decrease, for several hours. A similar result was also obtained in another series with potatoes, in which case the tubers were simply deeply incised, not actually cut through, and were then at once very tightly bound with soft string. At first a slight rise in the respiration

[1] l. c., Versuche, 13–15.

was noted, which, however, increased very suddenly on removal of the binding, at which time the cuts gaped widely open and so allowed free access of air to the injured parts. These experiments show that there can be hardly a doubt that the oxygen of the air plays an important part in the reaction of the respiratory functions of the plant towards injury.

Experiments made in atmospheres of hydrogen or of diminished percentage of oxygen substantiated those already described, and also showed some other facts. In Experiment 11 it will be seen that even in 6% oxygen the potatoes ceased to show the ordinary increase, but when finally exposed to air there was a gradual rising of the respiration-curve. In other experiments the percentage of O_2 was reduced to as low as four, which Stich[1] has shown may be safely used without complicating the results with intramolecular respiration. The decrease shown in Experiments 11 and 21 after exposure to an atmosphere of this composition for six hours is not the normal decrease due to the healing of the wounds, as the considerable increase after remaining over night in a current of air demonstrates. Corresponding experiments were made with carrots, but, although much the same phenomena were noted, they were not quite so clearly marked as in the former cases. In pure hydrogen it is shown that the intramolecular respiration is not so largely increased by injury (Expts. 9 and 10). After nine hours' exposure to hydrogen, the amount of CO_2 evolved was scarcely greater than the normal intramolecular respiration. In considering these experiments one must always remember the necessary accommodation which the functions of the plant have to undergo to the diminished pressure of the artificial atmosphere. On the other hand, it is shown by the experiments carried on with pure oxygen that a surplus of this gas produces no effect in yet further increasing the production of CO_2, from which fact it would seem that simply the action of oxygen on the tissues is not alone responsible for the

[1] l. c., p. 11.

increased respiration. Experiments 8, 21 a, and 39 illustrate this point.

The employment of well-aerated tissues, which, either from their small size or looseness of structure, afford free access of oxygen to their parts, threw yet more light on the subject. As has already been mentioned, seedlings of *Vicia Faba*, in spite of the irregularities in respiration associated with their rapid growth, showed an increase of CO_2 upon injury. But better still for this purpose are leaves, of which, as will be seen, a number of different kinds were tried. In all cases there was a distinct increase, the maximum being reached a few hours after, or at least during the same day as, the injury; while by the next day, if the leaf still lived, the respiration returned to about the normal (Expts. 28 and 32). It will be noted in all the experiments, both with seedlings and with leaves, that there was no sudden increase during the first few hours after wounding. It is, then, apparent that the shock of the injury itself must also play a part in the rise of the respiration of injured plants, since these parts of plants, which are already well supplied with O_2, or from their nature cannot contain any considerable quantity of oxidizable substances, also react when wounded.

As regards the sudden evolution of CO_2 that is noticed immediately after injury, the numerous experiments which were made to clear up this point seem to show that the phenomenon is due to the escape of this gas, already absorbed or held in the tissue, in a physical rather than a chemical manner. As has already been stated, thin and loosely constructed tissues, and also small masses of a more compact nature, such as the hypocotyls of seedlings, showed no evidence of this temporary increase. It was only with bulkier tissues like potatoes and carrots where this was found: in tissues, that is, where their size made it possible that CO_2 might be contained in them. But this point is more conclusively demonstrated in Experiments 12 and 19, in which the plants were exposed to a vacuum at once after injury. If there were any CO_2 present, as such, in the tissue,

it would naturally come off under the reduced pressure, and such was found to have been the case: for, although determinations were made but half an hour after injury, the sudden increase and subsequent fall found under ordinary atmospheric pressure were not observed. When injured potatoes are transferred to hydrogen, there is at first a considerably greater evolution of CO_2 than some hours later; which, as Stich has pointed out, is due to a process of accommodation which the plant undergoes, but which may also partly be due to the cause mentioned above. In the relation of the CO_2 produced and O_2 absorbed during the first few hours after injury, further support is found for the opinion here expressed regarding the source of the excess of CO_2. It will be seen that, whilst the CO_2-production is very large, the absorption of O_2 during the first two hours is less than later, when the CO_2 evolved has greatly decreased. Reference to Experiment 37 will show this, where with potatoes the amount of CO_2 given off during the first two hours after injury was 2·19% of the air in the receiver, while but 2% of O_2 had been absorbed. Further, during the next two hours 1·83% of CO_2 was evolved to 2·5% of O_2 absorbed; whereas on the next day, when the CO_2-production was again above 2%, the figures stood: CO_2 produced 2·56%; O_2 absorbed 3·2%. In the experiments with carrots the same result was also obtained; first two hours, CO_2 3·71% to O_2 3·80%; second two hours, CO_2 2·35% to O_2 3·6%; third two hours, CO_2 3.7% to O_2 5·3% (Expt. 4).

To obtain some idea of the amount of CO_2 which may be contained in such a large mass of tissue as a potato or a carrot, a number of experiments were made by which the contained gas was driven off by boiling water. The amount of CO_2 thus obtained was about 5 mg. to every gram of substance (Expts. A—G). The method is open to the objection that perhaps some of the CO_2 driven off may have been formed by the decomposition by the heat of organic compounds in the plant; but without much doubt a large portion of the gas was simply the contained or absorbed CO_2

in the tissues, as is indicated by the fact that practically all of it came off at once on warming. It was only desired to determine in a general way if there was much or little CO_2, as such, in the tissues, and not to arrive at a definite quantitative result. That there appears to be a considerable amount agrees with the other experiments discussed, and supports the view, already expressed, of the source of the first rush of CO_2 immediately after injury.

It was also desired to find if an increase of injury brought about a corresponding increase of respiration. As is shown in Experiments 6 and 18, there is a certain increase when the plant is further wounded, both in the case of potatoes and carrots, but one that does not bear any distinct relation to the amount of injury done. As before, leaves give an opportunity of more or less eliminating the question of aeration, and allowing a better means of observing the effect of the irritation alone. The leaves of *Veronica* and *Acacia* (Expt. 32) were experimented with from this standpoint. The latter was the better, although the former also served to show the reaction to some extent. With the *Acacia* the large compound leaves were taken from the plant, and determinations were made until it was found that the injury caused by the cutting of the petioles had passed its maximum. The pinnae were then cut off, and the main stalks once or twice cut across; as a result, the respiration rose a second time to a slightly higher point. When the evolution of CO_2 was once more on the wane, the leaves were injured for a third time, and more severely than before, by stripping off the pinnules and longitudinally slitting all the stalks. On this occasion the increase was much more strongly marked, and finally stopped on the gradual death of the leaves. This experiment shows then quite plainly that a certain effect is produced by increase of injury, although it must be remembered that in this case it was not possible to wash away the injured cells as was always done with longer tissues.

An examination of the results obtained with the apparatus already described for the comparison of the amount of CO_2

produced and of O_2 absorbed shows that the ratio of $\frac{CO_2}{O_2}$ does not change to any very great extent after injury, always excepting the first two hours, concerning which an explanation has already been given. The average ratio with uninjured potatoes is almost ·50, from which after the first sudden rise a second slower ascent is found, which corresponds to the curve of respiration as found in the Pfeffer-Pettenkofer apparatus. At the time of the greatest evolution of CO_2 it is about ·75 or ·80, falling again as the respiration decreases. In other words, somewhat more CO_2 is given off in proportion to the O_2 absorbed in the injured than in the uninjured condition. With the carrots experimented upon, even less change was found in three of the experiments (Expts. 40, 41, 42); it remained almost the same, while in a fourth it was slightly lower. In the analyses of respiration from seedlings, in two cases there was at first a rise in the ratio to which it kept during the entire experiment (Expts. 44 and 46), while in one case there was an appreciable fall to an amount equalling the respiration in the uninjured condition (Expt. 45). In the case of leaves, those of *Veronica speciosa* showed a distinct increase of the ratio at the time when the respiratory activity is at its maximum. In no case was there observed such a marked increase of the amount of oxygen absorbed as is indicated by the results of Stich's experiments.

It is apparent that the amount of CO_2 produced bears a close relationship to the amount of O_2 absorbed, the latter always, with the one exception already noted, being in excess of the former, but to no very great extent. It will be noticed in the following table that the amount of oxygen absorbed after injury, over and above that theoretically required for the amount of CO_2 produced, is somewhat more than in the uninjured condition, being at its highest when the respiration is strongest. It is, however, proportionally to the CO_2 formed, far less than before injury. It may possibly be due to the fact that the processes, involving the absorption of oxygen, which go on in the plants under normal conditions are simply somewhat

hastened by the freer access of the air to the tissues. The following table is calculated out from three of the experiments given in detail at the end of this paper. With the temperature and the ordinary pressure under which these experiments were carried out, 1 cc. of CO_2 may be called 1·8 mg. in weight, while the same amount of O_2 would be 1·3 mg., this being accurate within the limits of error of the methods employed and within the purposes of the table. In Experiments 37 and 42, where determinations were made immediately after injury, the amount of O_2 required for the CO_2 given off was not estimated for these two hours; for, as has already been explained, the relation between the two gases is then not normal. The object of this table is twofold: both to bring more clearly before the eye the exact amount of the gases interchanged, and to show that this method coincides as to the production of CO_2 with the direct determinations made with BaO_2H_2. Taking weight for weight, it will be seen that the two methods agree well as to the amount of CO_2 produced per hour. All the other experiments of the kind may be calculated out in a similar way from the data given.

	No. of Det.	Duration of Expt.	cc CO_2 produced.	cc O_2 absorbed.	mg. CO_2 produced.	mg. O_2 absorbed.	Amount in mg. O_2 required theoretically for CO_2 produced.	Amount in mg. O_2 absorbed not used in respiration.
Expt. 36	*a*	2 hours	2·27	4·92	4·10	6·40	3·00	3·40
8 potatoes	*b*	,,	11·00	11·72	19·80	19·20	14·40	4·80
195 grams	*c*	,,	13·96	18·45	25·20	24·00	18·35	5·65
	d	,,	15·74	19·68	28·30	25·60	20·60	5·00
	e	,,	12·61	16·50	22·70	21·20	16·50	4·70
Expt. 37	*a*	2 hours	2·55	5·61	4·60	7·30	3·35	3·95
8 potatoes	*b*	,,	11·77	10·20	20·10	13·30	...	...
200 grams	*c*	,,	9·33	13·77	16·80	18·00	12·70	6·30
	d	,,	13·06	16·36	23·40	21·20	17·00	4·20
	e	,,	11·48	15·05	20·35	19·60	14·40	5·20
Expt. 42	*a*	2 hours	9·10	12·84	16·40	16·70	11·95	4·75
2 carrots	*b*	,,	19·85	20·33	35·70	26·40	...	...
165 grams	*c*	,,	12·57	19·26	22·60	25·00	16·45	8·55
	d	,,	19·80	28·35	35·60	36·80	25·90	10·90
	e	,,	25·68	34·24	46·20	44·50	33·60	10·90
	f	,,	15·78	22·47	28·40	29·20	20·70	8·50

General Considerations.

Considering the increased respiration attending the injury of plants as an indication of stimulated activity on the part of the tissues, its significance is understood at once. In common with the general tendency of living organisms, plants endeavour to tide over the critical period following an injury by exerting an unusual effort to overcome the same. If the unfavourable conditions be too severe or too long-continued, death will naturally result; but until vitality is so far weakened that even ordinary activity is diminished, or until the crisis is past and recovery assured, evidences of an acceleration of the ordinary functions of life will pretty surely be found in almost any living organism. In the case of plants, so far as the kind of injuries produced by cutting are concerned, a far greater amount can be suffered without fatal effects than in even fairly lowly organized animals, so that a direct comparison between the two is not to be instituted. Nevertheless, even in such organs as leaves, where death of the parts follows comparatively rapidly on any severe injury, a distinct increase in the rate of respiration was found. But it is not necessary to be limited to only this type of abnormal conditions, for numerous authors have shown that plants respond by increased respiration, and hence, it is to be understood, increased activity in general, to other unnatural conditions. Elfving[1], experimenting with various plants under the influence of chloroform and ether, found that respiration was very considerably accelerated, although he did not determine the curve from the point of view of time. Johannsen's[2] work on the influence of an increased pressure of oxygen on seedlings shows a similar result even more plainly. He found that at first in almost every case the respiration was increased, falling again until finally death ensued. As has already been referred to, Stich[3]

[1] Öfversigt af Finska Vetensk.-Soc.'s Förh., Bd. xxviii, 1886.
[2] Untersuch. aus dem Bot. Inst. zu Tübingen, Bd. i, 716, 1885.
[3] l. c.

also discovered that, in a diminished quantity of oxygen, plants require a period of accommodation to the new conditions, during which time the respiration rises perceptibly. All of these results show, in common with what has been described here, that, under conditions which are unnatural, plants strive to rid themselves of, or surmount, the irritating influences of this unnatural state by a temporary increase of their vital energy.

In considering respiration as a measure of such increased activity, it must be remembered that in all these cases, and in the case of the work herein described, the methods necessarily employed show only the total effect of the injury upon the plants concerned. That is to say, it is impossible to locate the active region, to say whether it really is the whole potato, for instance, or only the part near the wound, which is affected, and which therefore respires more rapidly. In considering other effects of injury, Hauptfleisch[1] has shown that the movement of the protoplasmic contents of the cells is greatly increased in the neighbourhood of a wound, and that the activity so produced is transmitted to other cells after the lapse of a certain time. That this stimulation is carried to all the cells of the entire plant would hardly be supposed, nor does it seem to be the case from what Hauptfleisch records. In some comparatively delicate water-plants, as *Elodea* and *Vallisneria*, it was indeed seen to be propagated for a considerable distance; but in examples of hardier tissues, such as those of *Zea Mais* or *Triticum*, it seems to be much more restricted. In the experiments recorded here, indications of a more or less restricted zone of activity are suggested by the ever-increasing rate of respiration which followed successively severer injuries. That there may be a connexion between the phenomena which Hauptfleisch describes as 'secondary streaming' and the increased rate of respiration after injury is not at all improbable, in that they are all but part and parcel of the general stimulation of the normal functions of the plant.

[1] Pring's Jahrb., Bd. xxiv, p. 191 et seq., 1892.

That there are yet other forms of irritation to which plants respond by increased activity goes without saying. The familiar examples of the effect of many plant-parasites on their hosts show that this may become evident from even a morphological standpoint; and in other cases, where no direct atrophy or hypertrophy results, it would no doubt be found that the host-plant endeavours to counteract the effect of the parasite in such a way. Böhm [1] mentions the fact that the respiration of potatoes affected with *Phytophthora* is above the normal. Of course in such cases the period of stimulation is more prolonged than by simple wounding, for the source of irritation is always present, and also increasing; but before the plant succumbs there must be a time when the effect of the stimulus given by the parasite has reached a maximum.

Different as they may be, these examples all serve to illustrate the power of reaction against untoward and abnormal conditions which plants possess in common with other living organisms.

Statement of Conclusions.

To recapitulate in brief the conclusions arrived at from the experiments here recorded, as far as they can be judged from the plants employed, they may be stated as follows:—

1. That after injury to plant-tissue there results a greatly increased respiration, varying in intensity and duration with the character of the tissue involved and with the extent of the wounding. This increased activity of respiration, after reaching —usually within two days—a maximum, falls gradually, as the wounds heal over, to a normal or to an almost normal rate.

2. That this increased respiration may be ascribed to an effort on the part of the plant to recover from the injury by which the ordinary functions of the plant are stimulated, thereby demanding and necessitating an increased supply of oxygen.

[1] Bot. Centralblatt, Bd. l, p. 202, 1892.

3. That in large bulky tissues there is in the natural condition a certain amount of enclosed or absorbed CO_2, some of which is given off very suddenly during the first two or three hours after injury, thereby indicating a seemingly higher respiratory activity than in the hours which immediately follow.

4. That, in the plants experimented with, the ratio of the absorption of O_2 and production of CO_2 does not vary within very wide limits before and after injury, though there is a distinct, if small, increase in the proportion of CO_2 given off in the latter case. Also, that the amount of O_2 absorbed is always in excess of the amount theoretically required for the quantity of evolved CO_2.

BOTANISCHES INSTITUT, LEIPZIG,
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TABLES OF EXPERIMENTAL RESULTS.

A.

Determinations with Pettenkofer-apparatus. Air washed with potassic hydrate U-tubes. Hydrogen or oxygen when used washed also with potassic permanganate and mercuric chloride tubes. Air-current regulated by aspirator and gasometer in all experiments being 3·500 or a few cc. over per hour. When new experiments were begun, or a change made in the gases run through the receiver, the apparatus was always run at least a half-hour, and often longer, before a determination was made. Temperature of receiver and plants therein kept constant within ·5 to 1° by immersion in water. The first two or three determinations, unless it is stated otherwise in the description of experiment, were taken in the uninjured condition.

Experiment 1.

24 small potato-tubers, weight 200 grams. Air-current, 3·5 litres per hour. Ran thirty minutes before first determination.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
xi. 10. '95	9.45 – 10.45 a.m.	21°	2·00
,,	10.45 – 11.45 ,,	21°	1·70
Potatoes cut in two pieces lengthwise.			
xi. 10. '95	12.15 – 1.15 p.m.	21°	10·20
,,	1.15 – 2.15 ,,	21°	4·00
,,	2.15 – 3.15 ,,	20°·8	4·20
,,	3.15 – 4.15 ,,	20°·8	3·60
,,	4.15 – 5.15 ,,	20°·7	7·40
xi. 11. '95	9.25 – 10.25 a.m.	21°	10·00
,,	10.25 – 11.25 ,,	21°	10·60
,,	11.25 – 12.25 p.m.	20°·7	12·10
,,	12.25 – 2.25 ,,	20°·6	14·60*
,,	2.25 – 3.25 ,,	21°·1	14·50
,,	3.25 – 4.25 ,,	21°	16·30
,,	4.25 – 5.25 ,,	21°	14·70
xi. 12. '95	10.10 – 11.10 a.m.	20°·5	13·20
,,	11.10 – 12.10 p.m.	20°·7	13·30
,,	12.10 – 1.10 ,,	20°·7	11·10
,,	1.10 – 3.10 ,,	20°·6	11·00*
,,	3.10 – 4.10 ,,	20°·8	10·90
xi. 13. '95	10.00 – 11.00 a.m.	20°·9	10·70
xi. 14. '95	9.15 – 10.15 ,,	21°	7·70
xi. 18. '95	8.15 – 9.15 ,,	21°	4·20

* Reduced to value of 1 hour.

Experiment 2.

26 small potato-tubers, 200 grams. Respiration, uninjured, practically nothing. Sliced in half. Ran apparatus of half-hour. Air-current, 3·5 litres.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
xi. 15. '95	10.15 – 11.15 a.m.	20°	8·80
,,	11.15 – 12.15 p.m.	20°·5	8·10
,,	12.15 – 1.15 ,,	20°·5	6·90
,,	1.15 – 2.15 ,,	20°·7	6·90
,,	2.15 – 3.15 ,,	21°	6·50
,,	3.15 – 4.15 ,,	21°	6·70
,,	4.15 – 5.15 ,,	21°	7·70
,,	5.15 – 6.15 ,,	20°·8	7·70
,,	6.15 – 7.15 ,,	20°·8	6·30
,,	7.15 – 9.15 ,,	20°·6	8·85
xi. 16. '95	12.00 – 1.00 ,,	21°	11·70
,,	1.00 – 3.00 ,,	21°	10·25*
,,	3.00 – 4.00 ,,	21°·1	10·10
,,	4.00 – 5.00 ,,	21°·1	8·25
xi. 17. '95	10.30 – 11.30 a.m.	20°·5	9·10
,,	11.30 – 12.30 p.m.	20°·5	9·00
xi. 18. '95	9.30 – 10.30 ,,	20°·5	7·30
xi. 19. '95	9.20 – 10.20 ,,	20°·7	6·70
xi. 20. '95	3.50 – 4.50 p.m.	20°·8	5·70
xi. 21. '95	12.00 – 1.00 ,,	20°·4	4·50

* Reduced to value of 1 hour.

EXPERIMENT 3.

9 potato-tubers, 300 grams. Ran for two hours in uninjured condition, but only found a trace of CO_2 evolved. Air-current, 3·5 litres. Tubers quartered, washed, and dried, and at once tied together again with soft string.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
xi. 18. '95	1.45 – 2.45 p.m.	21°	16·50
,,	2.45 – 4.15 ,,	21°	13·20*
,,	4.15 – 5.15 ,,	20°·5	13·30
,,	5.15 – 6.15 ,,	20°·5	14·30
,,	6.15 – 7.15 ,,	20°·5	15·10
,,	7.15 – 9.15 ,,	20°·8	16·25*
xi. 19. '95	10.30 – 11.30 a.m.	20°·5	2·50
,,	11.30 – 12.30 p.m.	20°·3	20·50
,,	12.30 – 2.30 ,,	20°·5	16·10*
,,	2.30 – 3.30 ,,	21°	16·10
,,	3.30 – 4.30 p.m.	21°	16·30
,,	4.30 – 5.30 ,,	20°·8	16·50
,,	5.30 – 6.30 ,,	20°·5	17·30
xi. 20. '95	8.20 – 10.20 a.m.	20°·8	17·30
,,	10.20 – 11.20 ,,	20°·8	16·90
Removed bindings and separated the cut quarters.			
xi. 20. '95	11.40 – 12.40 p.m.	20°·9	22·00
,,	12.40 – 2.40 ,,	20°·9	20·00*
,,	2.40 – 3.40 ,,	20°·8	20·90
,,	5.00 – 6.00 ,,	20°·5	17·90
,,	6.00 – 7.00 ,,	20°·2	17·30
xi. 21. '95	10.00 – 11.00 a.m.	21°	14·10
xi. 29. '95	3.05 – 4.05 p.m.	21°	9·40
,,	4.05 – 5.05 ,,	20°·8	7·40
xi. 30. '95	4.00 – 6.00 ,,	21°	6·30*

* Reduced to value of 1 hour.

Experiment 4.

8 potato-tubers, 300 grams. Give only a trace of respiration in uninjured condition. Tubers quartered, washed, and dried. Air-current, 3·5 litres per hour.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
xi. 22. '95	9.15 – 10.15 a.m.	20°	13·40
,,	10.15 – 11.15 ,,	20°	9·00
,,	11.15 – 12.15 p.m.	20°·5	11·60
,,	12.15 – 1.15 ,,	20°·5	11·80
,,	1.15 – 3.15 ,,	20°·7	14·40*
,,	3.15 – 4.15 ,,	20° 6	14·80
,,	4.15 – 5.15 ,,	20°·8	15·70
,,	5.15 – 6.15 ,,	20°·6	16·00
,,	6.15 – 7.15 ,,	20°·5	16·80
xi. 23. '95	9.15 – 10.15 a.m.	20°·8	15·60
,,	10.15 – 11.15 ,,	20°·6	15·60
,,	11.15 – 12.15 p.m.	20°·8	16·00
,,	12.15 – 1.15 ,,	21°·0	18·60
,,	1.15 – 3.15 ,,	20°·7	18·40*
xi. 24. '95	11.30 – 12.30 ,,	20°·5	13·60
xi. 29. '95	6.15 – 7.15 ,,	21°	7·40
xii. 3. '95	8.15 – 9.15 a.m.	20°·8	3·20
xii. 4. '95	8.15 – 9.15 ,,	20°·8	1·60

Experiment 5.

11 potatoes, 300 grams. Cut into eight pieces. The object of this experiment was to verify the previous observations regarding the production of CO_2 during the first few hours after injury. Air-current, 3·5 litres.

Date.	Time.	Temp.	mg CO_2 produced per hour.
ii. 20. '96	9.40 – 10.40 a.m.	20°·8	51·20
,,	10.40 – 11.40 ,,	20°·7	36·00
,,	11.40 – 12.40 p.m.	20°·7	23·00
,,	12.40 – 2.10 ,,	20°·6	21·40*
,,	2.10 – 3.10 ,,	20°·4	21·80
,,	3.10 – 4.10 ,,	21°·0	21·60
,,	4.10 – 5.10 ,,	20°·8	23·40
,,	5.10 – 6.10 ,,	20°·8	23·60
ii. 21. '96	9.00 – 10.00 a.m.	20°·7	30·00
,,	10.00 – 11.00 ,,	20°·7	33·60

* Reduced to value of 1 hour.

EXPERIMENT 6.

8 potatoes, 300 grams. Halved, washed, and dried. Experiment to determine if there is any increase of respiration with increase of injury. Air-current, 3·5 litres.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
xii. 11. '95	8.20 – 9.20 a.m.	21°	6.20
,,	9.20 – 11.20 ,,	20°.8	8.60*
,,	11.20 – 12.20 p.m.	20°.7	8.50
Cut each half into six pieces, washed and dried. Time, 12.30.			
xii. 11. '95	2.20 – 3.20 p.m.	20°.7	21.70
,,	3.20 – 4.20 ,,	20 .6	20.70
,,	4.20 – 5.20 ,,	21°	27.40
xii. 12. '95	9.30 – 10.30 a.m.	21°	27.60

EXPERIMENT 7.

7 potatoes, 225 grams. Respiration in uninjured condition less than 1 mg. per hour. Quartered, washed, and dried, and then at once fastened the quarters together with clay. Air-current, 3·5 litres.

Date.	Time.	Temperature.	mg. Co_2 produced per hour.
xi. 28. '95	9.15 – 11.15 a.m.	20°.6	3.60*
,,	11.15 – 1.15 p.m.	20°.5	4.20*
,,	1.15 – 3.15 ,,	20°.5	6.60*
,,	3.15 – 5.15 ,,	20°.5	7.30*
xi. 29. '95	9.25 – 11.25 a.m.	21	7.60*
xii. 2. '95	2.35 – 4.35 p.m.	20°.4	2.50*
Clay removed.			
xii. 2. '95	5.05 – 6.05 p.m.	20°	24.60
,,	6.05 – 7.05 ,,	20°	25.40
,,	7.15 – 9.15 ,,	20°.3	20.40*
xii. 3. '95	6.10 – 7.10 ,,	20°.4	13.00
xii. 6. '95	9.20 – 10.20 a.m.	20°.3	12.00
xii. 12. '95	11.45 – 12.45 p.m.	20°.6	11.60

* Reduced to value of 1 hour.

EXPERIMENT 8.

6 potatoes, 310 grams. To determine effect of oxygen. Oxygen from gasometer 94 %. Before taking determinations with O a strong current was run through the apparatus until practically pure O came off. Current, 3·5 litres per hour.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.	Medium.
xii. 8. '95	9.00 – 11.00 a.m.	20°·4	2·10*	Air
,,	3.00 – 5.00 p.m.	20°·5	2·40*	Oxygen
Potatoes quartered, washed, and dried. First determination, 1¼ hours after injury.				
xii. 9. '95	10.00 – 11.00 a.m.	20°·5	10·60	Air
,,	11.00 – 12.00 m.	20°·5	13·80	Air
,,	12.00 – 1.00 p.m.	20°·3	13·60	Air
,,	1.00 – 3.00 ,,	20°·8	13·50*	Air
,,	3.40 – 4.40 ,,	20°·5	16·60	Oxygen
,,	4.40 – 5.40 ,,	20°·5	19·80	Oxygen
xii. 10. '95	9.30 – 10.30 a.m.	20°	21·40	Air
,,	11.00 – 12.00 m.	20°·8	23·40	Oxygen
,,	12.00 – 1.00 p.m.	20°·6	21·40	Oxygen
,,	1.20 – 3.20 ,,	20°·5	21·35	Air
,,	3.20 – 5.20 ,,	20°·4	22·00	Air*

* Reduced to value of 1 hour.

Experiment 9.

8 potatoes, 260 grams. Air- and hydrogen-current as before, 3·5 litres per hour.

Date.	Time.	Temperature.	Medium.	mg. CO_2 produced per hour.
i. 6. '96	4.00 – 5.00 p.m.	20°·6	Air	2·80
,,	5.00 – 7.00 ,,	20°·6	Hydrogen	3·10
,,	7.00 – 9.00 ,,	20°·4	,,	2·00
The potatoes were cut in half, washed as usual, and placed as quickly as possible in an atmosphere of hydrogen. Hydrogen run through apparatus in a strong stream until practically pure.				
i. 7. '96	10.00 – 11.00 a.m.	20°·8	Hydrogen	16·80
,,	11.00 – 1.00 p.m.	20°·6	,,	13·20*
,,	1.00 – 3.15 ,,	20°·7	,,	10·00*
,,	3.15 – 4.15 ,,	20°·5	,,	9·00
,,	4.15 – 5.15 ,,	20°·3	,,	8·90
,,	5.15 – 6.15 ,,	20°·2	,,	8·20
,,	6.15 – 7.15 ,,	20°·7	,,	7·90
,,	7.15 – 9.15 ,,	20°·7	,,	7·80*
The determinations of Jan. 7 are all in hydrogen, of the following day in air.				
i .8. '96	10.07 – 12.07 p.m.	20°·8	Air	12·80
,,	12.07 – 1.07 ,,	20°·6	,,	14·80
,,	1.07 – 3.07 ,,	20°·4	,,	15·80*
,,	3.07 – 5.07 ,,	20°·3	,,	15·70
i. 9. '96	10.00 – 11.00 a.m.	20°·4	,,	18·30
,,	11.00 – 12.00 m.	20°·5	,,	20·00
,,	12.00 – 1.00 p.m.	20°·4	,,	20·40
,,	1.00 – 3.00 ,,	20°·2	,,	18·80
,,	3.00 – 5.00 ,,	20°·5	,,	18·40*
,,	5.00 – 7.00 ,,	20°·5	,,	18·60*
i. 10. '96	11.00 - 1.00 ,,	20°·4	,,	10·30*
,,	1.00 – 3.00 ,,	20°·3	,,	11·00*
,,	3.00 – 5.00 ,,	20°·7	,,	10·60*
,,	5.00 – 7.00 ,,	20°·5	,,	8·40*

* Reduced to value of 1 hour.

Experiment 10.

8 potatoes, 250 grams. Quartered, washed, and dried. Evacuated under air-pump for half an hour, ran hydrogen in. Current, 3·5 litres per hour.

Date.	Time.	Temperature.	Medium.	mg. CO_2 produced per hour.
ii. 28. '96	8.20 – 9.20 a.m.	20°·8	Hydrogen	3·60
,,	9.20 – 10.20 ,,	20°·8	,,	3·80
,,	10.20 – 11.20 ,,	20°·7	,,	3·10
,,	11.20 – 12.20 p.m.	20°·5	,,	4·20
,,	12.20 – 1.20 ,,	20°·3	,,	5·40
,,	1.20 – 2.20 ,,	21°	,,	4·60
,,	2.20 – 3.30 ,,	20°·9	,,	4·10
,,	3.30 – 4.20 ,,	20°·7	,,	3·90
,,	4.20 – 5.20 ,,	20°·7	,,	3·20
,,	5.20 – 6.20 ,,	20°·4	Air	4·20
,,	6.20 – 7.20 ,,	20°·3	,,	4·50
,,	7.20 – 9.20 ,,	20°·3	,,	4·10*
ii. 29. '96	9.10 – 10.10 a.m.	20°·8	Hydrogen †	8·00
,,	10.10 – 11.10 ,,	20°·8	,,	8·20
,,	11.10 – 12.10 p.m.	20°·7	,,	6·00
,,	12.10 – 1.10 ,,	20°·6	,,	4·40
,,	1.10 – 2.10 ,,	20°·7	,,	4·20
,,	2.10 – 3.10 ,,	20°·7	,,	3·80
,,	3.10 – 4.10 ,,	20°·5	,,	3·80
,,	4.10 – 5.10 ,,	20°·5	,,	4·00
,,	5.10 – 6.10 ,,	20°·4	,,	3·60
,,	6.10 – 7.10 ,,	20°·6	Air	5·90
,,	7.10 – 9.20 ,,	20°·3	,,	8·70*
iii. 1. '96	8.20 – 9.20 a.m.	20°·8	,,	19·20
,,	9.20 – 10.20 ,,	20°·7	,,	22·40
,,	10.45 – 11.45 ,,	20°·7	Hydrogen †	10·00
,,	11.45 – 12.45 p.m.	20°·4	,,	8·10
,,	12.45 – 1.45 ,,	20°·4	,,	8·30
,,	1.45 – 2.45 ,,	20°·7	,,	7·60
,,	2·45 – 3.45 ,,	20°·6	,,	6·30
iii. 2. '96	9.00 – 10.00 a.m.	20°·9	Air	24·60
,,	10.00 – 11.00 ,,	20°·8	,,	23·10

* Reduced to value of 1 hour.

† In replacing air by hydrogen the receiver was evacuated under the air-pump several times, and a strong stream of hydrogen run in.

Experiment 11.

9 potatoes, 300 grams. Respiration in normal condition both in air and 60% oxygen very slight. Quartered, washed, &c. Air 6%, and 4% oxygen, used in this experiment. Current, 3·5 litres per hour.

Date.	Time.	Temperature.	Medium.	mg. CO_2 produced per hour.
ii. 10. '96	9.10 – 10.10 a.m.	20°·5	6 % Oxygen	24·30
,,	10.10 – 11.10 ,,	20°·5	,,	17·60
,,	11.10 – 12.10 p.m.	20°·4	,,	15·40
,,	12.10 – 1.10 ,,	20°·3	,,	12·00
,,	1.10 – 2.10 ,,	20°·6	,,	13·00
,,	2.10 – 3.10 ,,	20°·6	,,	12·80
,,	3.10 – 4.10 ,,	20°·5	,,	12·70
,,	4.10 – 5.10 ,,	20°·3	,,	16·00
,,	5.10 – 7.10 ,,	20°·3	,,	15·80
,,	7.10 – 9.10 ,,	20°·1	,,	18·40*
ii. 11. '96	10.00 – 11.00 a.m.	20°·7	Air	22·40
,,	11.00 – 12.00 ,,	20°·6	,,	21·60
,,	12.15 – 1.15 p.m.	20°·6	4 % Oxygen†	21·20
,,	1.15 – 2.15 ,,	20°·5	,,	22·00
,,	2.15 – 3.15 ,,	20°·5	,,	22·40
,,	3.15 – 4.15 ,,	20°·4	,,	15·40
,,	4.15 – 5.15 ,,	20°·5	,,	15·00
,,	5.15 – 6.15 ,,	20°·3	,,	14·80
ii. 12. '96	9.00 – 10.00 a.m.	20°·8	Air	32·80
,,	10.00 – 11.00 ,,	20°·8	,,	29·80

* Reduced to value of 1 hour.

† Receiver exhausted three times with 4% oxygen.

Experiment 12.

5 potatoes, 180 grams. Quartered and washed. Exhausted under air-pump for half an hour; from thence attached receiver at once to Pettenkofer apparatus.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
ii. 13. '96	4.00 – 5.00 p.m.	20°	8·40
,,	5.00 – 6.00 ,,	20°	9·00
,,	6.00 – 7.00 ,,	20°·4	9·25
,,	7.00 – 9.00 ,,	20°·3	9·40
ii. 14. '96	8.20 – 9.20 a.m.	20°·3	9·60
,,	6.00 – 7.00 p m.	20°·6	14·20

Experiment 13.

7 potatoes, 200 grams. Cut into quarters Feb. 11, 10 a.m.; allowed to stand over a day until the respiration had about reached its maximum. Air-current, 3·5 litres per hour.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
ii. 12. '96	8.00 – 9.00 a.m.	20°·8	17·60
,,	9.00 – 10.00 ,,	20°·7	18·00
Cut quarters covered with clay and stuck together.			
ii. 12. '96	12.30 – 1.00 p.m.	20°·4	10·40*
,,	1.00 – 3.00 ,,	20°·4	10·50*
,,	3.00 – 4.00 ,,	20°·9	12·00
,,	4.00 – 5.00 ,,	20°·7	13·40
ii. 13. '96	9.20 – 10.20 a.m.	21°	9·20
,,	10.20 – 11.20 ,,	21°	8·80
Clay removed.			
ii. 13. '96	11.45 – 12.45 p.m.	20°·8	23·20
,,	12.45 – 1.45 ,,	20°·6	19·84
,,	1.45 – 2.45 ,,	20°·5	15·90
,,	2.45 – 5.00 ,,	20°·5	16·20*

* Reduced to value of 1 hour.

EXPERIMENT 14.

5 carrots, 320 grams. Respiration in uninjured condition first determined. The wounds caused by the original gathering of the carrots had completely healed, and to accustom them to the conditions of the laboratory they had in this, as in all subsequent classes, been kept in the room for at least three days before use. Air-current, 3·5 litres per hour.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
xi. 25. '95	8.25 – 9.25 a.m.	20°·5	18·40
,,	9.25 – 10.25 ,,	20°·5	18·20
Cut carrots into quarters longitudinally. Washed and dried the cut surfaces.			
xi. 55. '95	10.38 – 11.38 a.m.	21°	64·60
,,	11.38 – 12.38 p.m.	21°	46·40
,,	12.38 – 2.38 ,,	20°·8	38·95*
The quarters tightly bound together again with soft string.			
xi. 25. '95	3.10 – 4.10 p.m.	20°·6	27·60
,,	4.10 – 5.10 ,,	20°·5	34·20
,,	5.10 – 6.10 ,,	20°·9	42·10
,,	6.10 – 7.10 ,,	20°·7	43·00
xi. 26. '95	9.20 – 10.20 a.m.	20°·9	42·65
,,	10.20 – 11.20 ,,	21°·0	46·10
,,	11.20 – 12.20 p.m.	21°·0	51·40
,,	12.20 – 1.20 ,,	20°·8	52·30
,,	1.20 – 2.50 ,,	20°·7	51·90*
,,	2.50 – 3.50 ,,	20°·4	50·10
,,	3.50 – 4.50 ,,	20°·2	45·50
,,	4.50 – 5.50 ,,	20°·8	50·00
,,	5.50 – 6.50 ,,	20°·5	52·80
xi. 27. '95	9.38 – 10.38 a.m.	21°·0	51·40
,,	10.38 – 11.38 ,,	20°·8	50·60
,,	12.15 – 1.15 p.m.	20°·7	53·20
,,	1.15 – 3.15 ,,	20°·5	55·00*
,,	3.15 – 4.15 ,,	21°·0	54·10
,,	4.15 – 5.15 ,,	21°·0	52·40
,,	5.15 – 6.15 ,,	20°·8	45·40
xi. 29. '95	11.30 – 12.30 ,,	20°·6	29·40
,,	12.30 – 2.30 ,,	20°·3	29·20*
xii. 2. '95	12.35 – 2.35 ,,	21°·0	23·70*

* Reduced to value of 1 hour.

Experiment 15.

4 carrots, 280 grams. Air-current, 3·5 litres per hour.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
xii. 30. '95	3.00 – 4.00 p.m.	21°	17·40
,,	4.00 – 5.00 ,,	22°	18·20
,,	5.00 – 6.00 ,,	20°·8	17·30
Each carrot cut in quarters longitudinally. Washed and dried.			
xii. 31. '95	8.00 – 9.00 a.m.	21°	56·60
,,	9.00 – 10.00 ,,	20°·7	43·40
,,	10.00 – 11.00 ,,	20°·6	33·70
,,	11.00 – 12.00 m.	20°·6	34·10
,,	12.00 – 1.00 p.m.	20°·5	36·50
,,	1.00 – 2.00 ,,	20°·4	34·40
,,	2.00 – 3.00 ,,	21°·0	38·20
,,	3.00 – 4.00 ,,	20°·8	38·40
,,	4.00 – 5.00 ,,	20°·8	40·70
,,	5.00 – 6.00 ,,	20°·7	41·00
,,	6.00 – 7.00 ,,	20°·4	40·80
,,	7.00 – 9.00 ,,	20°·4	42·60*
i. 1. '96	10.30 – 11.30 a.m.	21°·0	51·10
,,	11.30 – 12.30 p.m.	21°·0	53·40
,,	12.30 – 2.00 ,,	20°·7	53·80*
,,	2.00 – 3.00 ,,	20°·7	55·30
,,	3.00 – 4.00 ,,	20°·8	57.10
,,	4.00 – 5.00 ,,	20°·6	57·00
,,	5.00 – 6.00 ,,	20°·5	55·40
i. 2. '96	9.10 – 10.10 a.m.	20°·9	59·70
,,	10.10 – 11.10 ,,	20°·8	62·40
,,	11.10 – 12.10 p.m.	20°·8	58·25
,,	12.10 – 1.10 ,,	20°·5	56·10
,,	1.10 – 3.10 ,,	20°·3	53·70*
i. 3. '96	8.30 – 9.30 a.m.	21°	42·50
i. 4. '96	5.00 – 6.00 ,,	20°·7	29·70
i. 6. '96	9.15 – 10.15 ,,	21°	26·40
i. 10. '96	9.05 – 10.05 ,,	21°	21·20

* Reduced to value of 1 hour.

EXPERIMENT 16.

4 carrots, 300 grams. From same lot as carrots used in previous experiment. Air-current, 3·5 litres per hour.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
xi. 30. '95	8.15 – 9.15 a.m.	21°	20·20
,,	9.15 – 10.15 ,,	21°	19·60
	Cut in five pieces transversely.		
xi. 30. '95	10.45 – 11.45 a.m.	21°	29·4
,,	11.45 – 12.45 p.m.	20°·7	23·0
	Cut surfaces covered with clay.		
xi. 30. '95	2.30 – 3.30 p.m.	20°·5	20·15
,,	3.30 – 4.30 ,,	21°	22·60
,,	4.30 – 5.30 ,,	21°	23·80
xii. 1. '95	10.30 – 11.30 a.m.	21°	21·00
,,	11.30 – 12.30 p.m.	20°·8	24·40
xii. 2. '95	8.57 – 9.57 a.m.	20°·7	18·00
	Clay removed.		
xii. 2. '95	10.25 – 11.25 a.m.	20°·8	30·00
,,	11.25 – 12.25 p.m.	20°·4	25·20
xii. 5. '95	6.00 – 7.00 ,,	20°·3	24·40
xii. 12. '95	9.30 – 10.30 a.m.	21°	17·80

Experiment 17.

4 carrots, 290 grams. As in previous experiment. Air-current, 3·5 litres per hour. Cut in five transversely, and at once covered cut surfaces with clay.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
xii. 12. '95	9.30 – 10.30 a.m.	20°·8	20·30
,,	10.30 – 11.30 ,,	20°·6	21·35
,,	11.30 – 12.30 p.m.	20°·6	22·60
,,	12.30 – 2.30 ,,	20°·4	22·10*
,,	2.30 – 3.30 ,,	20°·5	24·00
xii. 13. '95	9.30 – 10.30 a.m.	20°·8	20·00
xii. 14. '95	4.00 – 5.00 p.m.	20°·2	19·30
	Clay removed.		
xii. 14. '95	5.00 – 6.00 p.m.	20°·6	35·20
,,	6.00 – 7.00 ,,	20°·5	32·00
,,	7.00 – 9.00 ,,	20°·5	33·40*
xii. 15. '95	10.10 – 12.10 ,,	21°	25·35
xii. 18. '95	6.10 – 7.00 ,,	20°·7	17·50

Experiment 18.

4 carrots, 290 grams. To determine if respiration increases with increase of injury. Air-current, 3·5 litres per hour. Cut in three pieces transversely.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
xi. 16. '95	1.00 – 3.00 p.m.	20°·5	20·20*
,,	3.00 – 4.00 ,,	20°·5	21·00
,,	4.00 – 5.30 ,,	20°·4	22·80*
	Cut each piece in four longitudinally.		
xi. 17. '95	10.52 – 11.52 a.m.	20°·2	28·80
,,	11.52 – 12.52 p.m.	19°·7	27·80
,,	12.52 – 2.52 ,,	20°·6	29·50*
,,	5.20 – 6.20 ,,	20°·5	32·40

* Reduced to value of 1 hour.

Experiment 19.

3 carrots, 200 grams. Quartered each longitudinally, placed under air-pump for three quarters of an hour. Air-current, 3·5 litres per hour.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
ii. 14. '96	3.00 – 4.00 p.m.	21°	27·30
,,	4.00 – 5.00 ,,	20°·8	28·00
,,	5.00 – 6.00 ,,	20°·8	29·20
,,	6.00 – 7.00 ,,	20°·6	29·40
,,	7.00 – 9.00 ,,	20°·5	31·90*

Experiment 20.

4 carrots, 260 grams. Mode of operation as in the experiments with potatoes in which hydrogen was employed. Air-current, 3·5 litres per hour.

Date.	Time.	Temperature.	Medium.	mg. CO_2 produced per hour.
i. 3. '96	10.10 – 11.10 a.m.	20°·0	Air	18·80
,,	12.20 – 1.20 p.m.	20°·0	Hydrogen	21·00
,,	1.20 – 3.20 ,,	19°·9	,,	19·60
,,	3.20 – 4.20 ,,	20°·6	,,	19·20
,,	4.20 – 5.20 ,,	20°·6	Air	15·80
,,	5.20 – 6.20 ,,	20°·5	,,	19·40
Each carrot cut longitudinally in quarters, Jan. 3, 6.30 p.m.				
i. 4. '96	9.22 – 10.22 a.m.	20°·8	Air	33·60
,,	10.22 – 11.22 ,,	20°·8	Hydrogen	31·80
,,	11.22 – 12.22 p.m.	20°·6	,,	33·40
,,	12.22 – 2.30 ,,	20°·5	,,	32·60*
,,	2.30 – 3.30 ,,	20°·4	,,	32·80
,,	3.30 – 4.30 ,,	20°·3	,,	30·20
,,	4.30 – 5.30 ,,	20°·9	,,	24·30
,,	5.30 – 6.30 ,,	20°·7	,,	21·20

* Reduced to value of 1 hour.

Experiment 21.

3 carrots, 270 grams. Quartered each longitudinally, and put as soon as possible in an atmosphere of 4% oxygen.

Date.	Time.	Temperature.	Medium.	mg. CO_2 produced per hour.
ii. 14. '96	9.05 – 10.05 a.m.	20°.3	4% O.	52.40
,,	10.05 – 11.05 ,,	20°.3	,,	40.80
,,	11.05 – 12.05 p.m.	20°.3	,,	32.00
,,	12.05 – 1.05 ,,	20°.2	,,	28.60
,,	1.05 – 2.05 ,,	20°.1	,,	27.30
,,	2.05 – 3.05 ,,	20°.1	,,	27.65
,,	3.05 – 4.05 ,,	20°.0	,,	28.30
,,	4.05 – 5.05 ,,	19°.9	Air	29.00
,,	5.05 – 6.05 ,,	19°.9	,,	29.20
,,	6.05 – 7.05 ,,	19°.6	,,	29.20
,,	7.05 – 9.05 ,,	19°.6	,,	29.80*
ii. 15. '96	9.35 – 10.35 a.m.	20°.4	4% O.	32.00
,,	10.45 – 11.15 ,,	20°.4	,,	32.80*
,,	11.15 – 12.15 p.m.	20°.4	,,	34.20
,,	12.15 – 1.15 ,,	20°.3	,,	32.00
,,	1.15 – 2.15 ,,	20°.3	,,	28.40
,,	2.15 – 3.15 ,,	20°.2	,,	26.20
,,	3.15 – 4.15 ,,	20°.6	,,	28.40
,,	4.15 – 5.15 ,,	20°.5	,,	25.60
,,	5.15 – 6.25 ,,	20°.5	,,	24.00*
ii. 16. '96	10.00 – 11.00 ,,	20°.3	Air	34.00

* Reduced to value of 1 hour.

EXPERIMENT 21 a.

3 carrots, 330 grams. Air-current, 3·5 litres per hour.

Date.	Time.	Temperature.	Medium.	mg. CO_2 produced per hour.
xii. 6. '95	10.35 – 11.35 a.m.	20°	Air	15.2
	Ran for 40 min. in oxygen current.			
xii. 6. '95	12.15 – 1.15 p.m.	20°	Oxygen	15·4
	Cut carrots in quarters longitudinally.			
xii. 6. '95	1.20 – 2.20 p.m.	20°·7	Air	55·6
,,	2.20 – 3.20 ,,	20°·6	,,	32·6
,,	3.20 – 4.20 ,,	20°·6	,,	28·8
	Ran for 45 min. in oxygen current.			
xii. 6. '95	5.00 – 6.00 p.m.	20°·5	Oxygen	28·4
,,	6.00 – 7.00 ,,	20°·7	,,	31·5
	Ran in an air-current overnight.			
xii. 7. '95	8.45 – 9.45 p.m.	20°·5	Air	38·4
	Ran for some time in oxygen.			
xii. 7. '95	10.15 – 11.15 a.m.	20°·5	Oxygen	36·4

Experiment 22.

4 red beets (*Beta vulgaris*), 250 grams. Had been in laboratory four days before use. Air-current, 3·5 litres per hour.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
xii. 4. '95	9.05 – 10.05 a.m.	20°	7·80
,,	10.05 – 11.05 ,,	20°	8·30
Quartered longitudinally. Pieces washed and dried.			
xii. 4. '95	11.35 – 12.35 p.m.	20°	18·20
,,	2.35 – 3.35 ,,	20°·3	17·00
,,	5.00 – 6.00 ,,	20°·5	15·10
xii. 5. '95	6.00 – 7.00 a.m.	20°·9	16·70
,,	9.10 – 10.10 ,,	21°·0	21·50
,,	10.10 – 11.40 ,,	21°·0	27·10*
,,	11.40 – 12.40 p.m.	20°·8	22·60
,,	12.40 – 2.40 ,,	20°·7	23·60*
,,	2.40 – 3.40 ,,	20°·5	22·80
,,	3.40 – 4.40 ,,	20°·5	23·40
,,	4.40 – 5.40 ,,	20°·3	22·20
xii. 7. '95	12.10 – 1.10 ,,	20°·5	14·80
,,	1.10 – 3.10 ,,	20°·3	14·60
xii. 8. '95	10.35 – 11.35 a.m.	20°·8	14·00
,,	11.35 – 12.35 p.m.	20°·8	15·30
xii. 9. '95	5.35 – 7.00 ,,	21°	10.60*
xii. 12. '95	12.30 – 2.30 ,,	20°·6	10·40*

* Reduced to value of 1 hour.

Experiment 23.

2 sugar beets (*Beta vulgaris*, var.), 300 grams. Had been in laboratory three days before use. Air-current, 3·5 litres per hour.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
xii. 18. '95	10.30 – 11.30 a.m.	20°·7	16·40
,,	11.30 – 12.30 p.m.	20°·6	17·00
Cut each beet in four longitudinally. Washed and dried cut surfaces.			
xii. 18. '95	1.30 – 3.30 p.m.	20°·5	30·20
,,	3.30 – 4.30 ,,	20°·5	22·16
,,	4.30 – 5.30 ,,	20°·5	26·40
xii. 19. '95	10.30 – 11.30 a.m.	21°·0	31·20
,,	11.30 – 1.00 p.m.	20°·6	28·30
,,	1.00 – 2.30 ,,	20°·5	28·30
,,	2.30 – 3.30 ,,	20°·3	24·60
,,	3.30 – 4.30 ,,	20°·7	24·40
xii. 20. '95	8.30 – 9.30 a.m.	21°·0	21·00
,,	9.30 – 10.30 ,,	20°·6	21·00
,,	10.30 – 11.30 ,,	20°·5	21·40
xii. 22. '95	10.00 – 11.00 ,,	20°·3	17·40

Experiment 24.

48 young seedlings of *Vicia Faba*. Hypocotyls from 2–4 cm. long. Weight, 110 grams. Air-current, 3·5 litres per hour.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
i. 11. '96	9.35 – 10.35 a.m.	20°·5	20·00
,,	10.35 – 11.35 ,,	20°·4	19·60
Hypocotyls split into shreds lengthwise without severing their connexion with the cotyledons.			
i. 11. '96	12.17 – 1.17 p.m.	20°·3	25·20
,,	1.17 – 2.17 ,,	20°·3	31·00
,,	2.17 – 3.17 ,,	20°·3	35·00
,,	3.17 – 4.17 ,,	20°·2	36·40
,,	4.17 – 5.17 ,,	20°·0	36·60
i. 12. '96	10.30 – 11.30 ,,	20°·6	39·40
,,	11.30 – 12.30 p.m.	20°·6	39·60
i. 13. '96	12.00 – 1.00 ,,	20°·4	24·90
,,	1.00 – 3.00 ,,	20°·9	22·80*
,,	3.00 – 4.00 ,,	20°·6	22·20
,,	4.00 – 6.00 ,,	20°·6	22·30*

* Reduced to value of 1 hour.

Experiment 25.

40 young seedlings of *Vicia Faba*. Hypocotyls from 1·5–3 cm. long. Weight, 80 grams. Air-current, 3·5 litres per hour.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
i. 17. '96	10.30 – 11.30 a.m.	20°·5	14·20
,,	11.30 – 12.30 p.m.	20°·4	14·30
Hypocotyls split into two longitudinally without severing their connexion with the cotyledons.			
i. 17. '96	1.15 – 3.15 p.m.	20°·00	15·60
,,	3.15 – 4.15 ,,	19°·50	15·60
,,	4.15 – 5.15 ,,	19°·40	16·10
,,	5.15 – 6.15 ,,	19°·40	17·30
,,	6.15 – 8.15 ,,	19°·60	20·00*
i. 18. '96	9.05 – 10.05 a.m.	20°·50	21·00
,,	10.05 – 11.05 ,,	20°·40	21·00
,,	11.05 – 12.05 p.m.	20°·30	22·10
,,	12.05 – 1.05 ,,	20°·80	23·20
,,	1.05 – 3.05 ,,	20°·60	27.10*
i. 19. '96	5.00 – 6.00 ,,	20°·60	13·20

Experiment 26.

45 young seedlings of *Vicia Faba*. Hypocotyls from 1–3 cm. long. Weight, 100 grams. Air-current, 3·5 litres per hour.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
i. 31. '96	9.10 – 10.10 a.m.	20°·7	29·00
,,	10.10 – 11.10 ,,	20°·6	30·00
About 1–2 millimetres cut off of the growing tips of the roots.			
1. '96	12.00 – 1.00 p.m.	20°·6	32·70
,,	1.00 – 3.10 ,,	20°·5	33·20*
,,	3.10 – 4.10 ,,	20°·8	36·40
,,	4.10 – 5.10 ,,	20°·7	39·30
,,	5.10 – 6.10 ,,	20°·4	42·00
ii. 1. '96	10.00 – 11.00 a.m.	20°·4	33·20
,,	11.00 – 12.00 m.	20°·7	32·80
,,	12.00 – 1.00 p.m.	20°·7	32·40
,,	1.00 – 2.00 ,,	20°·6	32·00
,,	2.00 – 3.00 ,,	20°·4	31·20
,,	3.00 – 4.00 ,,	20°·8	29·40
,,	4.00 – 5.00 ,,	20°·7	30·00

* Reduced to value of 1 hour.

Experiment 27.

82 seedlings of *Cucurbita Pepo.* Hypocotyls from 2–3 cm. long. Weight, 37 grams. Air-current, 3·5 litres per hour.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
i. 19. '96	9.35 – 10.35 a.m.	20°.6	15·40
,,	10.35 – 11.35 ,,	20°.5	16·00
The entire hypocotyl, so far as possible, split longitudinally.			
i. 19. '96	12.10 – 1.10 p.m.	20°.3	16·00
,,	1.10 – 3.10 ,,	20°.7	16·00*
,,	3.10 – 4.10 ,,	20°.7	16·20
,,	4.10 – 5.10 ,,	20°.6	16·40
,,	5.10 – 6.10 ,,	20°.6	16·80
i. 20. '96	9.55 – 11.55 ,,	20°.4	16·40*
,,	11.55 – 12.55 ,,	20°.8	22·00
,,	12.55 – 2.55 ,,	20°.8	22·20
,,	2.55 – 4.55 ,,	20°.6	21·00
,,	4.55 – 6.55 ,,	20°.5	26.35*
i. 21. '96	10.00 – 11.00 a.m.	21°.0	19·10
i. 22. '96	10.00 – 11.00 ,,	20°.7	15·70

Experiment 28.

50 young leaves of *Rhododendron calophyllum* from greenhouse. Weight, 90 grams. In dark. Air-current, 3·5 litres per hour.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
xi. 9. '95	10.15 – 11.15 a.m.	20°.6	3.20
,,	11.15 – 12.15 ,,	20°.4	3·60
Leaves cut transversely in thin strip about ·5 cm. wide.			
xi. 9. '95	3.10 – 4.10 p.m.	20°.3	6·60
,,	4.10 – 5.10 ,,	20°.7	7·20
,,	5.10 – 6.10 ,,	20°.5	7·10
xi. 10. '95	11.00 – 12.00 m.	20°.5	4·20

* Reduced to value of 1 hour.

Experiment 29.

7 young shoots of *Veronica speciosa* with about 80 leaves. Weight, 50 grams. Growing in green-house at temperature of 17°–20°. In dark. Air-current, 3·5 litres per hour.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
i. 27. '96	12.00 – 1.00 p.m.	20°·4	7·60
,,	1.00 – 3.00 ,,	20°·6	8·20
Leaves split four or five times longitudinally, but still remaining on petioles.			
i. 27. '96	4.00 – 5.00 p.m.	20°·4	16·20
,,	5.00 – 6.00 ,,	20°·4	19·00
,,	6.00 – 7.00 ,,	20°·3	15·80
i. 28. '96	9.00 – 10.00 a.m.	20°·8	10·30

Experiment 30.

8 young shoots of *Veronica speciosa* with about 95 leaves. Weight, 53 grams. In dark. Air-current, 3·5 litres per hour.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
i. 30. '96	9.30 – 10.30 a.m.	20°·6	7·60
,,	9.30 – 10.30 ,,	20°·5	7·80
Leaves split in four or five strips longitudinally, but still remaining on petioles.			
i. 30. '96	11.00 – 12.00 m.	20°·7	14·20
,,	12.00 – 1.00 p.m.	20°·4	16·00
,,	1.00 – 2.00 ,,	20°·3	15·00
,,	2.00 – 3.00 ,,	20°·8	11·00
ii. 1. '96	8.30 – 9.30 ,,	20°·8	8·60

EXPERIMENT 31.

52 grams of young leaves of *Viburnum odoratissimum* from green-house, the temperature of which was about 18°–20°. Light excluded from receiver. Air-current, 3·5 litres per hour.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
ii. 3. '96	10.37 – 11.37 a.m.	20°·4	11·40
,,	11.37 – 12.37 p.m.	20°·4	12·00
Leaves slit four or five times longitudinally, but pieces not actually separated.			
ii. 3. '96	1.10 – 2.10 p.m.	20°·2	16·00
,,	2.10 – 3.10 ,,	20°·2	17·20
,,	3.10 – 4.10 ,,	20°·5	15·40
,,	4.10 – 5.10 ,,	20°·4	15·40
ii. 4. '96	11.00 – 12.00 m.	20°·6	12·00
,,	4.00 – 5.00 p.m.	20°·6	12·00

EXPERIMENT 32.

50 grams of leaves of *Acacia lophocantha*, growing in green-house at temperature of about 25°. In dark. Air-current, 3·5 litres per hour. Determination first made of respiration excited by injury due to removal from stem.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
ii. 24. '96	10.00 – 11.00 a.m.	20°·3	14·00
,,	11.00 – 12.00 m.	20°·3	15·60
,,	12.00 – 1.00 p.m.	20°·2	15·80
,,	1.00 – 2.00 ,,	20°·2	15·30
,,	2.00 – 3.00 ,,	20°·5	18·00
,,	3.00 – 4.00 ,,	20°·5	17·20
,,	4.00 – 5.00 ,,	20°·4	16·40
,,	5.00 – 6.00 ,,	20°·2	15·60
,,	6.00 – 7.00 ,,	20°·2	15·40
Pinnae cut off and main stem of leaf cut in pieces.			
ii. 25. '96	10.10 – 11.10 a.m.	20°·4	17·00
,,	11.10 – 12.10 p.m.	20°·4	19·00
,,	12.10 – 1.10 ,,	20°·3	17·80
,,	1.10 – 3.10 ,,	20°·2	16·00*
,,	3.10 – 4.10 ,,	20°·5	14·80
Cut pinnules off and cut petioles lengthwise.			
ii. 25. '96	5.10 – 6.10 p.m.	20°·2	24·40
,,	6.10 – 7.10 ,,	20°·	24·80
,,	7.10 – 9.10 ,,	20°·4	21·30*

* Reduced to value of 1 hour.

EXPERIMENT 33.

60 grams of twigs of *Salix alba* in pieces about 10 cm. long. Had been gathered in the open, and kept in laboratory nine days before the determinations were made. Air-current, 3·5 litres per hour.

Date.	Time.	Temperature.	mg. CO_2 produced per hour.
i. 23. '96	8.55 – 9.55 a.m.	20°.8	11.40
,,	9.55 – 10.55 ,,	20°.6	12.80
,,	10.55 – 11.55 ,,	20°.6	12.16
Twigs split longitudinally in several pieces.			
i. 23. '96	1.30 – 2.30 p.m.	20°.4	17.30
,,	2.30 – 3.30 ,,	20°.8	17.80
,,	3.30 – 4.30 ,,	20°.4	17.40
,,	4.30 – 5.30 ,,	20°.3	17.20
,,	5.30 – 6.30 ,,	20°.2	17.20
,,	6.30 – 8.00 ,,	20°.3	17.60*
i. 24. '96	11.07 – 12.07 ,,	20°.6	17.00
,,	12.07 – 1.07 ,,	20°.5	18.10
,,	1.07 – 3.07 ,,	20°.4	19.50*
,,	3.07 – 5.07 ,,	20°.2	16.80*
i. 27. '96	9.15 – 11.15 a.m.	20°.2	16.90*

* Reduced to value of 1 hour.

B.

In the experiments made to determine the relation between the amount of CO_2 produced and of O_2 absorbed, the time interval was always two hours. The apparatus used and the method of analysis of the air has already been described[1]. For the purpose of these experiments the air of the laboratory was perfectly pure, containing but a bare trace of CO_2. The amount of O_2 in the air is taken in the following tables at

[1] See this paper, page 534 et seq.

the round figure of 20·8 %. The amount of O_2 absorbed by the plants is calculated by the diminution in per cent. contained in the air in receiver at close of experiment, as given in the tables below.

	Capacity of receiver, cc.	Temp.	Volume of air taken.	CO_2 absorbed by KOH.	O_2 absorbed by Pyrogallol.	% CO_2 produced.	% O_2 absorbed by plants.	$\frac{CO_2}{O_2}$
EXPERIMENT 34.								
4 potatoes, 150 grms. 130 cc. Uninjured. i. 18. '96, 8.45–10.45 a.m.	700	19°·6	53·30	·30	10·65	·60	·80	·75
Same, i. 18. '96, 10.45–12.45 p.m.	,,	19°·4	58·50	·40	11·60	·70	1·00	·70
Same, quartered at 1 p.m. i. 18. '96, 3–5 p.m.	,,	19°·6	55·20	1·05	10·20	1·95	2·40	·81
Same, i. 19. '96, 9.30–11.30 a.m.	,,	19°·7	51·30	1·20	9·25	2·35	2·75	·85
EXPERIMENT 35.								
8 potatoes, 200 grms. 190 cc. Uninjured. i. 24. '96, 10–12 m.	700	19°·8	53·40	·20	10·50	·40	1·10	·36
Same, i. 24. '96, 1–3 p.m.	,,	19°·6	53·80	·30	10·60	·56	1·10	·50
Same, quartered, washed, &c. i. 24, '96, 5.15–7.15 p.m.	,,	19°·2	51·30	·80	9·40	1·55	2·50	·62
Same, i. 25. '96, 1–3 p.m.	,,	19°·7	54·30	1·40	9·60	2·60	3·10	·84
Same, i. 25. '96, 3.05–5.05 p.m.	,,	20°	54·80	1·30	9·90	2·35	2·70	·87
EXPERIMENT 36.								
(*a*) 8 potatoes, 195 grms. 180 cc. Uninjured. i. 30. '96, 9.30–11.30 a.m.	800	20°·	54·40	·20	10·90	·37	·80	·46
(*b*) Quartered at 12 m. First determination, i. 30. '96, 3.10–5.10 p.m.	,,	20°	50·30	·90	9·30	1·8	2·40	·75
(*c*) Same, i. 3. 96, 9.30–11.30 a.m.	,,	20°·2	52·90	1·20	9·40	2·27	3·00	·76
(*d*) Same, i. 31. '96, 1–3 p.m.	,,	20°·1	53·80	1·50	9·30	2·8	3·50	·80
(*e*) Same, ii. 3. '96, 1–3 p.m.	,,	20°	54·10	1·10	9·80	2·05	2·65	·77
EXPERIMENT 37.								
(*a*) 8 potatoes, 200 grms. 190 cc. Uninjured. ii. 3. '96, 8.50–9.50 a.m.	700	20°·3	52·50	·25	10·35	·50	1·10	·45
(*b*) Same, quartered; determination made at once; ii. 3. '96, 11–1 p.m.	,,	20°·1	54·90	1·20	10·10	2·20	2·00	1·10
(*c*) Same, ii. 3. '96, 3.20–5.20 p.m.	,,	20°·3	54·50	1·00	9·85	1·83	2·70	·68
(*d*) Same, ii. 4. '96, 11–1 p.m.	,,	20°·2	54·60	1·40	9·60	2·56	3·20	.80
(*e*) Same, ii. 6. '96, 11–1 p.m.	,,	20°·0	54·20	1·20	9·65	2·20	2·95	·75

	Capacity of receiver, cc.	Temp.	Volume of air taken.	CO_2 absorbed by KOH.	CO_2 absorbed by Pyrogallol.	% CO_2 produced.	% O_2 absorbed by plants.	$\frac{CO_2}{O_2}$
EXPERIMENT 38.								
6 potatoes, 200 grms. 190 cc. Uninjured. ii. 5. 96, 8.20–10.20 a.m.	700	20°·2	53·00	·30	10·50	·56	1·00	·56
Same, quartered; determination made at once, ii. 5. 96, 10.30–12.30 p.m.	,,	20°·3	53·90	1·20	9·60	2·22	3·00	·73
Same, ii. 5. '96, 1–3 p.m.	,,	20°·2	51·75	·75	9·40	1·45	2·65	·54
Same, ii. 5. '96, 3.10–5.10 p.m.	,,	20°·2	52·00	·90	9·20	1·80	3·10	·60
Same, ii. 6. '96, 1–3 p.m.	,,	20°·6	53·80	1·20	9·40	2·25	3·30	·68
Same, ii. 9. '96, 9–11 a.m.	,,	20°·2	51·10	1·00	9·15	1·95	2·90	·67
EXPERIMENT 39.								
7 potatoes, 205 grms. 180 cc. Uninjured. i. 7. '96, 1–3 p.m.	800	20°·4	53·30	·30	10·60	·56	·90	·62
Same current of O_2 run through apparatus until almost pure.	,,							
i. 28. '96, 9.45 a.m. Analysis of gas in receiver.	,,		**52.00**		**50.90**		**97.90**[1]	
i. 28. '96, 9.45–11.45 a.m. in O_2	,,	20°·2	54·00	·30	52·40	·55	·90	·61
Same, quartered, washed, and dried, in *air*, i. 28, '96, 1.30–3.30 p m.	,,	20°·1	53·80	·90	10·00	1·68	2·20	·77
Same, ran O_2 through. Analysis of air in receiver, i. 28. '96, 3.55 p.m.	,,		**52.30**		**51.20**		**98.10**[1]	
i. 28. '96, 3.55–5.55 p.m. in O_2	,,	20°·2	54·60	·90	52·30	16·5	2·30	·72
Same in air, i. 29. '96, 1–3 p.m.	,,	20°·0	54·80	1·10	9·60	2·55	3·30	·77
Same, ran O_2 through. Analysis of air in receiver, i. 29. '96, 4 p.m.	,,		**57.00**		**49.10**		**86.20**[1]	
i. 29. '96, 4–6 p.m. in O_2	,,	20°·3	56·00	1·35	46·40	2·40	3·30	·73
EXPERIMENT 40.								
2 carrots (*Daucus Carota*), 150 grms. 150 cc. Uninjured. i. 27. '96, 9.20–11.20 a.m.	700	20°	54·10	·70	10·30	1·30	1·75	·75
Same, at 12 m. cut longitudinally into quarters. i. 27. '96, 3.30–5.30 p.m.	,,	20°	55·00	1·00	10·00	1·80	2·60	·70
Same, i. 28. '96, 9.30–11.30 a.m.	,,	20°	53·70	1·10	9·60	2·05	2·90	·71
Same, i. 28. '96, 3.10–5.10 p.m.	,,	20°	52·50	1·30	8·90	2·48	3·80	·65
EXPERIMENT 41.								
2 carrots (*Daucus Carota*), 150 grms. 150 cc. Uninjured. ii. 1. '96, 10.40–12.40 p.m.	700	20°·3	51·10	·70	9·70	1·35	1·8	·75
Same; quartered longitudinally. Determination at once. ii. 1. '96, 12.50–2.50 p.m.	,,	20°·2	56·80	1·30	10·40	2·30	2·5	·92
Same, ii. 1. 96, 2.55–4.55 p.m.	,,	20°·3	50·75	·90	9·15	1·77	2·75	·65
Same, ii. 1. '96, 10–12 m.	,,	20°·2	54·90	1·40	9·40	2·55	3·70	·69

[1] % O in receiver at beginning of experiment.

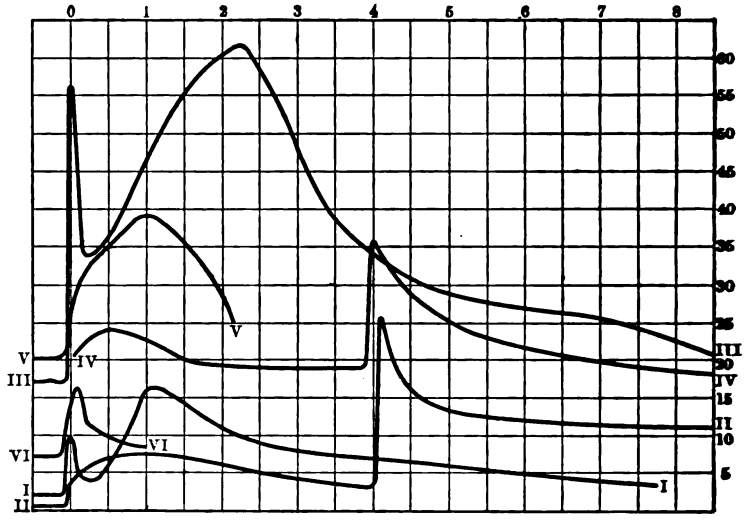
	Capacity of receiver, cc.	Temp.	Volume of air taken.	CO_2 absorbed by KOH.	O_2 absorbed by Pyrogallol.	% CO_2 produced.	% O_2 absorbed by plants.	$\frac{CO_2}{O_2}$
Experiment 42.								
(*a*) 13 carrots (*Daucus Carota*), 165 grms. 160 cc. Uninjured. ii. 14. '96, 10.50–12.50 p.m.	700	20°.1	52.70	.90	9.70	1.70	2.40	.71
(*b*) Same, quartered longitudinally; determination at once. ii. 14. '96, 1–3 p.m.	,,	20°.2	56.60	2.10	9.60	3.70	3.80	.97
(*c*) Same, ii. 14. 96, 3.05–5.05 p.m.	,,	20°.2	55.10	1.30	9.50	2.35	3.60	.65
(*d*) Same, ii. 14. 96, 7.05–9.05 p.m.	,,	20°.1	54.20	2.00	8.40	3.10	5.30	.70
(*e* Same, ii. 15. '96, 3.15–5.15 p.m.	,,	20°.2	51.10	2.45	7.35	4.80	6.40	.75
(*f*) Same, ii. 16. '96, 10–12 m.	,,	20°.3	51.00	1.50	8.50	2.95	4.20	.70
Experiment 43.								
2 carrots (*Daucus Carota*), 150 grms. 150 cc. Uninjured. ii. 18. '96, 8.45–10.45 a.m.	700	20°.3	54.50	.70	10.30	1.25	1.90	.66
Same, quartered longitudinally; determination at once. ii. 18. '96, 10.55–12.55 p.m.	,,	20°.2	56.40	1.60	9.90	2.85	3.25	.88
Same, ii. 18. '96, 1–3 p.m.	,,	20°.2	53.50	1.10	9.40	2.05	3.25	.63
Same, ii. 18. '96, 3.05–5.05 p.m.	,,	20°.1	57.10	1.50	9.80	2.65	3.30	.70
Same, ii. 19. '96, 2–4 p.m.	,,	20°.4	53.50	1.20	9.60	2.24	2.90	.77
Same, ii. 20. '96, 10–12 m.	,,	20°.1	52.20	.90	9.60	1.72	2.40	.72
Experiment 44.								
45 young seedlings *Vicia Faba*, hypocotyls 2–3 cm. long, 100 grm. 20 cc. Uninjured. ii. 3. '96, 9.30–11.30 a.m.	800	20°.4	53.60	1.00	9.20	1.86	3.60	.52
Same, root tips cut off, ii. 3. '96, 3.25–5.25 p.m.	,,	20°.2	55.10	1.20	9.70	2.20	3.20	.69
Same, ii. 4. '96, 9.45–11.45 a.m.	,,	20°	57.70	1.60	9.80	2.77	3.80	.73
Same, ii. 4. '96, 4–6 p.m.	,,	20°.3	57.50	1.40	10.00	2.45	3.55	.69
Experiment 45.								
42 young seedlings *Vicia Faba*, hypocotyls 2–3 cm. long, 100 grms. 100 cc., ii. 7. '96, 8.50–10.50 a.m.	700	20°.2	55.80	1.20	9.40	2.15	3.95	.55
Same, root tips cut off, ii. 7. '96, 11–1 p.m.	,,	20°.1	55.20	1.50	9.10	2.72	4.30	.63
Same, ii. 7. '96, 1.10–3.10 p.m.	,,	20°.3	52.20	1.50	8.70	2.90	4.10	.71
Same, ii. 7. '96, 4.10–6.10 p.m.	,,	20°.2	52.00	1.50	8.60	2.90	4.20	.70
Same, ii. 8. '96, 10.30–12.30 p.m.	,,	20°.3	55.40	1.00	9.70	1.80	3.30	.55

	Capacity of receiver, cc.	Temp.	Volume of air taken.	CO_2 absorbed by KOH.	O_2 absorbed by pyrogallol.	% CO_2 produced.	% O_2 absorbed by plants.	$\frac{CO_2}{O_2}$
EXPERIMENT 46.								
45 young seedlings *Vicia Faba*, hypocotyls 3–5 cm. long, 100 grms. 97 cc. Uninjured. ii. 10. '96, 10.40–12.40 p.m.	800	20°·0	53·70	1·00	9·15	1·87	3·80	·49
Same, roots transversely cut but not completely divided, ii. 10. '96, 1–3 p.m.	,,	20°·2	50·80	1·30	8·60	2·55	3·90	·65
Same, ii. 10. '96, 4–6 p.m.	,,	20°·3	53·10	1·50	8·90	2·80	4·05	·71
Same, ii. 10. '96, 7–9 p.m.	,,	20°·0	57·30	1·80	9·50	3·15	4·40	·72
Same, ii. 11. '96, 1–3 p.m.	,,	20°·3	54·70	1·50	9·20	2·90	3·95	·73
EXPERIMENT 47.								
7 shoots *Veronica speciosa*; about 130 leaves, 47 grms. 50 cc. Uninjured. i. 30. '96, 11–1 p.m.	800	20°·0	54·40	·80	10·10	1·50	2·25	·66
Same, leaves and buds cut lengthwise, i. 30. '96, 4.10–6.10 p.m.	,,	20°·0	54·20	1·20	9·70	2·20	2·90	·76
Same, i. 31. '96, 10–12 a.m.	,,	20°·0	56·60	1·10	10·20	1·95	2·80	·70
EXPERIMENT 48.								
7 shoots *Veronica speciosa*; about 140 leaves, 50 grms. 50 cc. Uninjured. ii. 12. '96, 10–12 p.m.	800	20°·2	55·80	·50	10·50	·90	2·00	·45
Same, ii. 12. '96, 1–3 p.m.	,,	20°·1	53·00	·70	10·00	1·30	1·90	·68
Same, ii. 13. '96, 10–12 p.m.	,,	20°·3	51·20	·30	10·10	·60	1·10	·55

C.

Experiments to determine amount of CO_2 contained in living potatoes and carrots. Driven off by boiling water and determined in Pfeffer-Pettenkofer apparatus.

		Weight objects.	mg. CO_2
With Potatoes.			
Expt. A.	3 potatoes	66 grams	36·0
,, B.	3 ,,	56 ,,	32·0
,, C.	5 ,,	105 ,,	61·2
,, D.	3 ,,	60 ,,	27·8
Average CO_2 per gram 5·5 mg.			
With Carrots (*Daucus Carota*).			
Expt. E.	2 carrots	102 grams	38·0
,, F.	1 ,,	95 ,,	34·4
,, G.	2 ,,	101 ,,	46·8
Average CO_2 per gram 3·7 mg.			



Woodcut 3.

I. Respiration-curve of Potato, cut, and allowed to run its normal course. See Experiment 1. II. Same, but cut surfaces covered with clay which was removed during the fifth day. See Experiment 7. III. Normal curve of injured Carrot. See Experiment 15. IV. Curve showing result of covering cut surfaces with clay as in II. See Experiment 17. V. Normal curve of injured seedlings (*Vicia Faba*). See Experiment 24. VI. Normal curve of injured leaf (*Veronica speciosa*). See Experiment 30. On the horizontal the days are numbered, beginning from time of injury. Before Zero is given the respiration in the uninjured condition. On the perpendicular the number of mg. CO_2 per hour are given.

Notes on Sugar-cane Diseases.

BY

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With Plate XXVI.

MASSEE'S paper on *Trichosphaeria Sacchari*[1] induced me to make a renewed investigation of some of the parasites affecting the sugar-cane in the island of Java. I shall give the results of this investigation here, at the same time as those formerly arrived at, but which till now had only been published in the Dutch language[2]. Mr. Barber forwarded to me alcohol specimens of diseased sugar-cane from the West Indies, and his kindness enabled me to determine the identity of several of the West Indian parasites of the cane with those existing in Java.

I shall begin first of all by describing two fungus-diseases, the red smut and the pineapple-disease.

I. Red Smut (*Colletotrichum falcatum*).

Red smut affects the interior of the stems of the sugar-cane. Externally no trace of its existence can be discovered, except when the canes are very badly attacked, in which case the

[1] Annals of Botany, Vol. vii. No. XXVIII, December, 1893.

[2] Went, Het Rood Snot; and Went, De Ananasziekte van het Suikerriet: Mededeelingen van het Proefstation West Java: both published in Archief voor de Javasuiker-industrie, I, 1893; also Bot. Centralbl., 59, 1894: also J. H. Wakker, De Ananasziekte of het Zwart Rot in Oost-Java, Mededeelingen van het Proefstation Oost-Java, N. Serie, No. 7; Archief voor de Javasuiker-industrie, 1894, p. 209.

[Annals of Botany, Vol. X. No. XL. December, 1896.]

leaves die: but as soon as the stems are split longitudinally, red spots are to be seen on the section. These spots are purple, but the colour is not equally distributed, it being darker in one place than in another. Very characteristic of this disease are white blotches in the interior of the red spots, these white blotches extending mostly in a direction transverse to the stalk. Another peculiarity is only to be seen on transverse sections, namely, that in the direction of the periphery the distinction between the dark red diseased tissue and the sound portion of the stem is very sharply defined. The periphery of the stem is generally not attacked; this explains the fact that the symptoms of the disease are not seen externally, because the vascular bundles communicating with the leaves at the top are not affected. These red spots are to be found either in the middle of a joint or near the node; but in either case careful examination is sure to show that the cane has been damaged in these places, either by some insect, like the moth-borer, or by some fungus-disease. Badly diseased canes are often red-spotted in the interior from the base to the top; sometimes brownish spots may be seen at the nodes.

With regard to the macroscopic characteristics of the disease, there only remains to be said that vascular bundles emerging from the diseased spots are red-coloured and gummy along a certain distance from the diseased place. I may here take the opportunity to state that wherever a part of the stem of a sugar-cane has been damaged, either by man or by animal or vegetable parasites, the vascular bundles communicating with the damaged spot become gummy and red-coloured. This is not only the case in Java, but from the diseased canes received from Mr. Barber I was able to conclude that sugar-cane in the West Indies behaves in exactly the same manner. A description of these gumming vascular bundles has been given by Valeton[1]. I would here repeat the statement that Bacteria have nothing to do with this

[1] Th. Valeton, Bijdrage tot de Kennis der Serehziekte: Mededeelingen van het Proefstation Oost-Java, 1891.

gumming [1], and I do so because I have several times read the assertion that I should have found the Australian gumming-disease, as described by Cobb [2], in Java. Now, only a few months ago I observed some phenomena here which might be identical with the Queensland disease, ascribed by Cobb to the influence of parasitic Bacteria. But the gummy red-coloured vascular bundles found in all diseases of the cane have nothing to do with this, and are not due to any action of Bacteria.

If we turn now to the microscopic examination of the disease, it will be seen that the cells in the diseased spots are filled with the mycelium of a fungus. The hyphae contain a great many small oil-drops, soluble in alcohol and ether. Frequently in older blotches the fungus can no longer be found in the centre of the diseased spots, but here the oil-drops very generally have persisted, so that the direction of the former hyphae is still manifest. It is very easy to cultivate this fungus by bringing sections of diseased canes into a damp place. In from twelve to twenty-four hours a beautiful mycelium grows out of the diseased spots; this mycelium is at first almost white, but afterwards its colour becomes greyish or smoky, perhaps between Nos. 2 and 39 of Saccardo [3]: if the hyphae are moistened, the colour changes and becomes light olive-green (No. 39, Saccardo). The mycelium is again characterized by its oil-drops. After a few days' growth chlamydospores are formed, sometimes in the middle of a filament (Fig. 3), but mostly at the end of a hypha (Figs. 1, 2). They contain large oil-drops and have a somewhat irregular form; their cell-wall is coloured dark olive-green.

In making cultures of the fungus on nutrient solutions or agar-agar, these chlamydospores are produced in great

[1] Went, De Serehziekte: Mededeelingen van het Proefstation West Java. Archief voor de Javasuikerindustrie, I, 1893; Bot. Centralbl. 59, 1894.

[2] N. A. Cobb, Plant Diseases and their Remedies; Diseases of the Sugar-cane. Department of Agriculture, N. S. W. Sydney, 1893.

[3] P. A. Saccardo, Chromotaxia seu Nomenclator Colorum. Ed. altera, 1894.

numbers, but very few conidia are to be found. In order to get these it is necessary either to seek in cracks of diseased canes or to allow a piece of longitudinally split diseased cane to dry slowly. In the latter case, after twenty-four to forty-eight hours, some black streaks appear running lengthwise of the cane on the cut surfaces. On examining these by the aid of the microscope, it is clear that they are stromata; from each stroma spring a great number of dark-brown hairs, and at the foot of these the conidia are found (Fig. 4). Figs. 5 and 6 give a more magnified view of two such hairs, with the conidia forming cells at their base. Each hair is divided into cells. Very often the case of Fig. 6 occurs, viz. that the top of the hair remains uncoloured. Fig. 7 represents three stages in the formation of conidia on their basidia; in *c* the conidium is almost ripe, and has already assumed its characteristic sickle-shaped form. This may be still better seen in Fig. 8, where a ripe conidium is represented; the conidium is colourless and contains a protoplast which is highly refringent. Fig. 9 shows some other forms of conidia, which sometimes may be found; the difference in form is mostly due to the position in which these conidia are seen. I have already remarked that in nutrient solutions very few conidia are developed; in that case there are no brown hairs to be found, or only here and there a single one. Fig. 10 shows a mycelium-filament of such a culture, which has given off several short branches; these will again develop branches, which will be the basidia on which the conidia are found.

The conidia germinate very easily in nutrient solutions; Fig. 11 shows three stadia of germinating conidia. By cultivating one single conidium in a hanging-drop, the evidence may easily be got that the mycelium emanating from it later on, forms the above-mentioned chlamydospores; the result therefore is that these and the conidia belong to the same fungus.

From the above-mentioned characters it will be seen that the fungus belongs to the genus *Colletotrichum*; I have named it *Colletotrichum falcatum* because of the shape of the conidia.

The fungus will be sufficiently recognizable I am sure if I give some measurements: hairs, 100–200 × 4 μ; conidia, 25 × 5 μ; basidia, (about) 20 × 8 μ (but the size of the basidia is very variable).

Every cane diseased by red smut contained the mycelium of *Colletotrichum falcatum*, and as during the last four years I have examined a very large number of diseased canes, it seemed very probable that this fungus might be the cause of the disease. But this had to be proved. With this object I made incisions in sound canes and inoculated them with mycelium from pure cultures of *Colletotrichum falcatum*; the wound was shut off from the air by a little tinfoil. After ten days some of the canes treated in this manner were cut longitudinally; it could be very distinctly seen that round the wound the cane had become diseased by red smut. After twenty days the whole joint was diseased, showing the characteristic red spots with white blotches, and under the microscope the cells were seen to be filled with the mycelium of *Colletotrichum falcatum*. Incisions made in sound canes, without inoculating with the fungus, did not produce the disease; only the dead cells round the wound became coloured red, as is always the case when a cell of the sugar-cane dies.

We may conclude therefore that *Colletotrichum falcatum* is the cause of red smut. But it is only a wound-parasite; sound canes, which have not been damaged, cannot be attacked by the fungus, with the exception of very young parts of the stem. This is practically of no importance, as these parts are protected by the surrounding sheaths of the leaves.

The attack of the fungus may cause serious damage, owing to the deterioration of the canes and the diminution of the sugar. At the Tjomal estate, where the disease was first detected in 1892, at the beginning of the grinding season samples are taken from all the various cane-fields and cut longitudinally; all the canes of those fields which prove to be attacked in any serious degree by red smut are reaped and crushed before the others, because the disease spreads very

soon over the canes that are still sound. It will be very difficult to combat the disease in any other way; it is impossible of course to remove and destroy the diseased canes, for we cannot tell by the outward appearance if canes are attacked by *Colletotrichum falcatum*. But of course everything may be done to preserve the cane from injury, more especially by borers.

As already stated, the disease was first detected in 1892 at the Tjomal estate; from thence it is spreading gradually over more estates, especially those situated to the west of Tjomal. Now it is very interesting that the fungus exists in other parts of Java, in those too where the disease is yet unknown. There *Colletotrichum falcatum* is a simple saprophyte on dead cane-leaves. Hence it seems that there are special circumstances which induce this saprophyte to become a wound-parasite.

The fungus has also been found outside of Java. Massee[1] attributes to it the so-called root-disease in the West Indies, but gives no evidence whatever for his opinion. I received the fungus from the West Indies, so I could identify it with *Colletotrichum falcatum* from Java; and according to my experience about the fungus and the disease it causes at Java (not only in the purple Java cane but in other varieties such as Louzier too) I think it extremely improbable that the so-called root-disease—a disease which looks very much like the 'sereh' in Java—is caused by *Colletotrichum falcatum*. As this fungus is generally a saprophyte, its mere presence on diseased canes is no evidence for its being the cause of the disease.

In conclusion, I may add that I have cultivated *Colletotrichum falcatum* on very different nutrient solutions for several years—since 1892; that I have searched for other forms of this fungus on diseased canes, but have never got anything but conidia and chlamydospores. Only in two cultures on agar-agar with cane-juice and ½% peptone did I get

[1] Bulletin of Miscellaneous Information, Royal Gardens, Kew, 1893, p. 347.

a yeast-form, but as I have not been able to get this in more cases, and as I have never seen under the microscope that conidia of *Colletotrichum falcatum* form yeast-cells, I think it is probable that these yeast-cells were an impurity which had somehow found its way into the cultures.

II. Pineapple-Disease (*Thielaviopsis ethaceticus*).

In most cases this disease only attacks cuttings, though it may be found in the stems of half-grown or full-grown cane too, if these are damaged; but this last mode of occurrence of the disease is very rare.

Diseased cuttings are blackish on the cut surface; on dividing them longitudinally the interior shows a red or crimson colour, if they are only slightly diseased, whereas the more serious symptoms are a black colour in the centre of the crimson-coloured part. When diseased cuttings are planted, or when the disease breaks out after the cuttings have been planted, the buds do not sprout, or die shortly after having sprouted. Very characteristic is the odour of diseased canes, as soon as they are cut; it reminds one of different fruits, especially pineapples (hence the name).

In examining the diseased tissue under the microscope, it will be seen that the red colour is occasioned by dead cells, of which the cell-walls have taken this colour; in the interior of these cells is to be found the colourless mycelium of a fungus. The black colour is occasioned by the conidia of this same mould.

It is very easy to cultivate the fungus; these conidia soon germinate in a drop of any nutrient solution; some stages of germinating conidia are represented in Fig. 12.

Cultures of the fungus remain snowy-white so long as only vegetative mycelium is developed; but very soon (twelve to twenty-four hours) afterwards the colour grows darker, somewhat olive-green or dark green (between Nos. 34 and 39 of Saccardo), in consequence of the formation of conidia. These conidia exist in two kinds, which I call

macro- and microconidia. The macroconidia are situated in apical chains at the end of short branches of the mycelium (Fig. 13). Their cell-walls are of a dark olive-green colour. Their contents are generally not to be seen, owing to the colouration of the cell-walls and to the number of large oil-drops in the interior (Fig. 14). Only where these oil-drops are very small and where the colouration of the cell-wall is lighter, as in Fig. 15, the protoplasm with the vacuoles may be visible. The conidium at the top of the chain very often—but not always—is almost spherical, as in Figs. 13 and 14, the other conidia being more elongated. In Fig. 16 the formation of such a chain is to be seen ; *a* is the beginning, one conidium has been formed. Under the microscope I was able to observe that the top of the hypha under the conidium formed a new transverse septum, and thus a new conidium was made: *b* was drawn one and a half hour after *a*; the top conidium had become larger and more spherical: three hours afterwards, in *c*, the apical conidium had become still larger and shows a commencing colour of the cell-wall; two new septa had been formed, being the beginning of the development of a third and a fourth conidium. It results from this that the macroconidia are formed in a basipetal manner.

The microconidia are also produced in chains, but they arise partly in the interior of the conidia-bearing-cell. Fig. 17 shows in *a* the very first beginning of a conidia-bearing hypha, which is a thick somewhat curved branch of the mycelium. This curve remains in the adult hyphae, as is to be seen in Fig. 17 *b* or Fig. 18. The number of conidia in a chain may be three or four, but generally is very great, Fig. 18 not being an extreme case at all. Fig. 19, much more magnified, may give an idea of the manner in which these microconidia are formed at the top of a hypha, from which they are afterwards extruded. Three of the conidia are quite free; one is just escaping from the cell-wall (*a*) of the hypha; another has just been formed, but its basal wall (*b*) still forms a part of the hypha; another one has its basal wall not yet quite developed (*c*). These microconidia

have a rectangular form and are colourless; sometimes they are slightly coloured and more oval, approaching in their form the macroconidia. Fig. 20 gives the tops of three hyphae bearing microconidia; in *a* the upper part is empty, the microconidia having been shed; in *b* there is one conidium just escaping from the hypha; in *c* two microconidia are not yet pushed out.

Macro- and microconidia may be found on branches of the same mycelium filament. In making cultures of macroconidia, both forms of conidia are obtained, just as well as when cultures are begun with microconidia; proving that they belong to the same fungus. Lastly, all sorts of transitory stages between the two forms of conidia may be found: as in Fig. 21, where *a* and *b* represent two stages in the typical formation of a chain of macroconidia, whereas in *c* it can be seen that the lowest of these macroconidia is being pushed out of the hypha in which it has been formed, just like a microconidium. The size of the microconidia and that of the macroconidia too (but in a less degree) is extremely variable, so that the measurements given here are only approximately true; macroconidia 16–19 × 10–12 μ; microconidia 10–15 × 3·5–5 μ; length of the microconidia-bearing hyphae 100–200 μ. In order to give an idea of the difference in form and size of the conidia I have delineated in Fig. 22 a group of conidia, as I found it under the microscope.

Though I made a great number of cultures of this fungus I could not detect other organs of reproduction; the same result was got by Wakker, who also has been cultivating the fungus at Pasuruan for some years. The microconidia of the fungus are similar to those described by Zopf for *Thielavia basicola*[1], but the macroconidia are very different. I therefore have given the fungus the generic name of *Thielaviopsis* and call the species in question *Thielaviopsis ethaceticus*.

It may be easily proved that *Thielaviopsis ethaceticus* is the cause of the pineapple-disease, by bringing some of the conidia

[1] W. Zopf, Ueber die Wurzelbräune der Lupinen, eine neue Pilzkrankheit. Zeitschrift für Pflanzenkrankheiten, Bd. i, 1891, p. 72.

on to the cut surface of cuttings of the sugar-cane. After two or three days the cuttings exhibit all the symptoms of the disease. The same may be seen by infecting half- or full-grown cane with the conidia of the fungus through holes previously made. The conidia cannot infect canes through the sound epidermis of the stem, with the exception of the very young white parts of it, which however are naturally protected by the surrounding leaf-sheaths. From these facts it follows that the means of preventing the disease are very simple. They consist in protecting from the air the cut surfaces of cuttings. This is done here by means of tar, previously rendered more liquid by the addition of a little arrack. On estates where this method is employed the disease has ceased to show itself.

The physiological properties of *Thielaviopsis* are very interesting. It is quite easy to cultivate the fungus on all sorts of nutrient solutions, on potatoes, bananas, &c. It is a saprophyte, which attacks all sorts of materials containing sugar, such as different fruits, mangoes, pineapples, bananas, &c. It spreads so easily that it is very difficult to get rid of the fungus where it once exists. In most of these cultures the agreeable odour can be detected. What is the substance possessing this flavour produced by the fungus? In order to determine this, I made a culture of *Thielaviopsis* in 1½ litre of a sterilized solution, containing 15% of saccharose, 1% of peptone, and a little K_3PO_4 and $MgSO_4$. After a week the liquor was distilled, and in this distillate the presence of ethyl acetate (and perhaps of very small quantities of other esters) and of ethylic alcohol could be proved. I further made a solution of 1% peptone in water with very little K_3PO_4, $MgSO_4$ and $CaCl_2$. I divided this solution into seven flasks, and added to them separately the following substances: No. 1, nothing; No. 2, cellulose; No. 3, starch; No. 4, 10% dextrine; No. 5, 10% glucose; No. 6, 10% saccharose; No. 7, 5% ethylic alcohol. After having sterilized I inoculated with the conidia of *Thielaviopsis*. In flasks Nos. 1, 2 and 3 the fungus developed pretty

well, but no odour was detected, whereas this was very strong and pronounced in Nos. 4–7. Thus *Thielaviopsis ethaceticus* can invert dextrine and saccharose into glucose, can make ethylic alcohol out of glucose, and finally can oxidize this alcohol to acetic acid. In old cultures of the fungus, however, the odour disappears, so that it looks as if the ethyl-acetate might afterwards be used and assimilated by the fungus.

A similar fungus has been described by Kayser[1], and afterwards by Dávalos and Acosta[2]. Kayser has found a yeast and a fungus, which both produce an ester, resembling in its odour the pineapple. The yeast is of no importance whatever here, but the fungus possesses, according to the description and the figure (though both are somewhat indistinct), the same kind of conidia as the microconidia of *Thielaviopsis*. Macroconidia are not described by Kayser, but the fungus produces ethyl-acetate and small quantities of ethylic alcohol. Other fungi producing conidia similar to the microconidia of *Thielaviopsis* are *Endoconidium* and *Pyxidiophora*.

III.

The paper by Massee on *Trichosphaeria Sacchari* gave me the impression that what he calls the macro- and microconidia of this fungus are similar to or very little different from the form which I had described as *Thielaviopsis ethaceticus*. This opinion was confirmed by the material I received from the West Indies containing so-called macro- and microconidia of *Trichosphaeria*, which could not be distinguished from my *Thielaviopsis*. This was an inducement for me to study Massee's paper very closely; for if his opinions were right, that these conidia are a form of *Trichosphaeria Sacchari* and that this fungus has a *Melanconium*-stage too, there would be an enormous probability that this would be the

[1] E. Kayser, Note sur les Ferments de l'Ananas. Ann. de l'Institut Pasteur, 1891, tome v, p. 456.

[2] Dávalos y Acosta, Nota sobra el fermento alcohólico de la piña. Crónica Médicoquirurgica de la Habana, 1892, No. 10.

case also with the Java fungus. The measures to prevent the pineapple-disease ought to be altered in that case.

Now it seems to me that Massee has not proved that these two forms of conidia belong to the same fungus as the *Melanconium* of the cane, nor that these three have an ascigerous stage in *Trichosphaeria Sacchari*. The reason why the experiments of Massee do not seem conclusive to me is, that he has neglected the first condition of any research into the development of fungi, which condition is, quoting the words of De Bary[1], 'Das erste Postulat einer morphologisch-entwickelungsgeschichtlichen Untersuchung (ist) der Nachweis der zu irgend einer Zeit nothwendig vorhandenen organischen Continuität successiver Entwickelungszustände, bei welcher also das später auftretende Glied als ein Theil des nächstfrüheren beginnt.'

Massee found two perithecia of *Trichosphaeria Sacchari* on much decayed canes received from Barbados, which sprung from a point that had previously borne a crop of microconidia. Massee calls the evidence in favour of a genetic connexion between the perithecia and the microconidia strong, where I might be inclined to speak of it as a very slight indication of the possibility of any such connexion. This supposition according to Massee was proved to be correct when young perithecia were found in a flask containing an old culture produced from a macroconidium. No other evidence whatever is given. Whilst I do not assert that these perithecia and the macroconidia do *not* belong together, I urge that there is no sufficient evidence to prove this.

Almost the same is the case with regard to the connexion between *Melanconium* and the macro- and microconidia. In one of three flasks containing cultures of *Melanconium*-conidia there developed the micro- and macroconidia resembling *Thielaviopsis*. The most probable explanation of this would have been that these macro- and microconidia were an impurity having by chance entered into the flask; and

[1] De Bary, Vergl. Morphologie u. Biologie der Pilze, Mycetozoen und Bacterien, 1884, p. 137.

this would be the more probable if they really are identical with *Thielaviopsis*, because this fungus spreads so very easily that almost any cane sent from Java, however sound it is or attacked by whatever disease, will contain this fungus. No other experiments are given by Massee in support of the view that these fungi are forms of the same species; for the experiment in which a small portion of diseased cane containing hyphae of the *Melanconium*-stage was introduced into a slit made in a healthy cane where afterwards macroconidia were produced, cannot prove anything, as of course it cannot be known what other organisms had been introduced with the diseased cane.

IV. Experiments with Melanconium-Stylospores.

I thought it necessary to try some experiments with *Melanconium*-stylospores. I may premise that *Melanconium Sacchari* (probably identical with what Cobb[1] calls *Strumella Sacchari*) is called in the West Indies 'rind-fungus.' According to a great many different publications by Barber, Bovell, Hart, Fawcett, &c.[2], and to the already mentioned paper of Massee, a serious disease of the cane is attributed to it. This same disease has been described by Cobb in Queensland; whereas Boname[3] states that *Melanconium Sacchari* in Mauritius attacks only dead canes. Now here in Java also I have found a *Melanconium* on the sugar-cane, which so much resembles the fungus from the West Indies that one might be inclined to consider them both as the same species. But the *Melanconium* in Java is only to be found on dead canes; it is only a saprophyte, and not a wound-parasite, as the form in the West Indies seems to be. Experiments, which I will describe hereafter, have brought me to this conviction. It may be possible that the *Melanconium* from

[1] Cobb, l. c., p. 23.

[2] Barbados, Report of Dodds Reformatory, 1892, 1893; Supplement to the Leeward Islands Gazette, 1893, xxiv; Bulletin, Botanical Department, Jamaica, 1894, 1895; Royal Botanic Gardens, Trinidad, Bulletin, 1894, 1895.

[3] Colony of Mauritius, Rapport de la Station Agronomique, 1894.

Java, notwithstanding this outward resemblance, is another species than the West Indian *Melanconium*. I think it however to be much more probable that both fungi belong to the same species, the one being the saprophytic, the other the wound-parasitic form,—somewhat as in the case of *Colletotrichum falcatum*. It is not quite certain, therefore, that I experimented with the same *Melanconium* as did Massee, but this is no reason why I should not give the results of my experiments.

I commenced by introducing in the usual way one *Melanconium*-stylospore into a hanging-drop of a nutrient solution; but I found that this method could not be followed, as most of the stylospores did not germinate, so that an enormous number of hanging-drops would have been necessary in order to get one mycelium. So I introduced a number of stylospores—about ten—into one hanging-drop, and in this manner I could observe germination in about 5% of the stylospores. But here another difficulty arose. Since these *Melanconium*-pycnids are only to be found on dead canes, spores of many other saprophytes are introduced into the hanging-drops at the same time. Spores of other Fungi could be eliminated, but Bacteria were a great nuisance, though the nutrient solution was made slightly acid. They were in so far a nuisance as they increased in number very fast and stopped the development of the young *Melanconium*-mycelia. This was the reason why I did not get ripe conidia in these hanging-drops, and only succeeded in obtaining them after washing the mycelium several times in a sterilized nutrient solution and then putting it on the surface of agar-agar (with nutrient substances). In this manner I was at last successful in getting pure cultures, without any Bacteria.

Fig. 23 shows the germination of two *Melanconium*-stylospores; sometimes two germ-tubes are developed, sometimes one, as in Fig. 23. Figs. 24 and 25 show a part of a mycelium developed from the *Melanconium*-stylospore *c* in different stages of development. This mycelium is beginning to form conidia but did not get beyond the stage of Fig. 25 (stopped by the

Bacteria mentioned above). However, these two figures will demonstrate that the mode of formation of the conidia is the same as in the agar-cultures. Parts of these have been drawn in Figs. 26, 27 and 28, and more highly magnified in Figs. 29, 30 and 31. It will be easily seen that the dark black spherical conidia arise by budding on the top of cells with a very refrangent protoplast, which are inflated somewhat like the top of the sporangial filament of *Pilobolus*. The conidia have a size of $16-14{\cdot}5 \times 13-12\,\mu$; they are often found on dead leaves of the cane. Fig. 32 is a chlamydospore formed in a culture of a *Melanconium*-stylospore in a hanging-drop; but these chlamydospores are very rare.

Melanconium-stylospores were introduced into slits made in sound canes; the mycelium developed in the dead cells surrounding the slits, but in no case (I made nine experiments) did they attack the healthy tissue of the cane. Exactly the same result followed when I used, not the *Melanconium*-stylospores, but the mycelium or the large black conidia. On dead leaves of the cane *Melanconium*-stylospores may germinate; afterwards the black spherical conidia are found on the same spot, but healthy leaves are not attacked by the fungus.

Pieces of sugar-cane were sterilized on the outside by keeping them for some time in a flame; they were then divided longitudinally with a sterilized knife and put into a sterilized glass box. On the cut surface I placed some *Melanconium*-stylospores. Very soon—within a few days—the spot where I had placed the stylospores became dark red, and a mycelium could be detected with the microscope in the interior of these red cells. Of the ten experiments which I made in this manner, seven were finally destroyed by Bacteria (a complete sterilization of the cane being almost impossible); but in three cases the mycelium developed through the cane as this was dying and gave rise on the surface under the epidermis to the pycnidia of *Melanconium*; but the stylospores were smaller in size than those which I gathered in the field, though on the other hand they germinated much more easily.

I got the same result by inoculating sterilized canes with the mycelium or with the large black spherical conidia. In operating with these conidia I succeeded, in two cases out of ten, in obtaining the formation of *Melanconium*-pycnidia on the surface of the cane, getting thus the complete evidence that these two forms—the stylospores and the large black conidia—belong to the same species. I ought to add that the *Melanconium*-stylospores never developed on sterilized canes unless I had previously inoculated these canes with the *Melanconium*-stylospores or with the mycelium developed from them, or with the large black spherical conidia. As the disease attributed to the rind-fungus does not yet exist here, I regret that I am not able to experiment with *Melanconium* from the West Indies, because I do not wish to introduce this fungus in the living state into Java, considering the danger of infection.

V. Summary.

1. *Colletotrichum falcatum*, being a saprophyte on the leaves of the sugar-cane, can become a wound-parasite under conditions still unknown, and is thus the cause of the disease of the cane at Java called Red Smut.

2. No evidence has been given up to the present that *Colletotrichum falcatum* is the cause of any other sugar-cane disease.

3. *Thielaviopsis ethaceticus* is a general saprophyte, behaving sometimes as a wound-parasite, and then causing the pine-apple-disease of the sugar-cane in Java.

4. There is some probability that the macro- and micro-conidia described by Massee as a form of *Trichosphaeria Sacchari* are identical with *Thielaviopsis*.

5. At present only micro- and macroconidia of *Thielaviopsis* are known.

6. Massee has not given sufficient evidence that the ascigerous stage, called *Trichosphaeria Sacchari*, and the macro- and microconidia, are forms of the same fungus.

7. The evidence given by Massee of these macro- and micro-

conidia and the *Melanconium*-stylospores belonging to the same fungus is insufficient.

8. The *Melanconium* which is found on dead canes in Java is no parasite; it lives only on canes which are already dead. It follows from this that the *Melanconium* from Java is perhaps different from that in the West Indies.

9. The stylospores of the Java *Melanconium* give rise, on germination, to a mycelium producing large black spherical conidia; these conidia placed on dead canes give again rise to the formation of the pycnidia of *Melanconium*.

KAGOK-TEGAL, *October*, 1895.

EXPLANATION OF FIGURES IN PLATE XXVI.

Illustrating Professor Went's paper on Sugar-cane Diseases.

Figs. 1–11. *Colletotrichum falcatum.*

Figs. 1–3. $\frac{2}{1}\frac{0}{0}$. Mycelium with chlamydospores (*g*). Figs. 1 and 2 the chlamydospores at the top of a hypha, Fig. 3 in the middle. Of the cell-contents only the oil-drops have been drawn.

Fig. 4. $\frac{8}{1}\frac{0}{0}$. Stroma (*str.*) with hairs and conidia (*c*).

Figs. 5, 6. $\frac{2}{1}\frac{0}{0}$. Young hairs from a stroma; at their bases are basidia, having formed in one case a conidium *c*.

Fig. 7. $\frac{2}{1}\frac{0}{0}$. Three different stages *a*, *b*, *c* in the formation of conidia on the top of the basidia.

Fig. 8. $\frac{2}{1}\frac{0}{0}$. A conidium.

Fig. 9. $\frac{2}{1}\frac{0}{0}$. Three differently-shaped conidia.

Fig. 10. $\frac{2}{1}\frac{0}{0}$. Mycelium giving off short branches which will produce the basidia.

Fig. 11. $\frac{2}{1}\frac{0}{0}$. Germinating conidia (*c*); *a*, one germ-tube is developing; *b* and *c*, with germ-tubes on both ends of the conidia.

Figs. 12–22. *Thielaviopsis ethaceticus.*

Fig. 12. $\frac{2}{1}\frac{0}{0}$. Macroconidia germinating.

Fig. 13. $\frac{2}{1}\frac{0}{0}$. Branch of the mycelium with a chain of six macroconidia.

Fig. 14. $\frac{2}{1}\frac{0}{0}$. Chain of three macroconidia with large oil-drops in the interior.

Fig. 15. $\frac{2}{1}\frac{0}{0}$. Chain of six macroconidia not yet fully grown, with very slightly coloured cell-walls and small oil-drops, so that the vacuoles are distinctly visible.

Fig. 16. $\frac{2}{1}\frac{0}{0}$. Successive stages of development of macroconidia: *a*, one conidium is visible; *b*, one and a-half hour later, under the first conidium, which has increased in size, a new one is developed; *c*, three hours later, a third and fourth conidium begin to develop; the first conidium becomes slightly coloured.

Fig. 17. $\frac{2}{1}$. *a*, a very young branch, which will give rise to a long curved hypha with microconidia, like that figured in *b*.

Fig. 18. $\frac{2}{1}$. Branch of the mycelium with chain of microconidia.

Fig. 19. $\frac{2}{1}$. Top of a branch with microconidia: *a*, top of the hypha where one conidium is just escaping; *c*, transverse cell-wall separating a microconidium, not yet free from the cell-wall of the surrounding hypha; *c*, transverse cell-wall not yet quite formed.

Fig. 20. $\frac{2}{1}$. Three apices of hyphae bearing microconidia: *a*, without conidium; *b*, with a conidium just escaping; *c*, with two conidia.

Fig. 21. $\frac{2}{1}$. Three stages of development of microconidia: *a*, with one conidium; *b*, three hours afterwards, with three quite developed conidia and one not yet ripe; *c*, one hour later, the last-formed basal conidium slides out of the cell-wall of the hypha like a microconidium.

Fig. 22. $\frac{2}{1}$. A group of macro- and microconidia of different size.

Figs. 23–32. *Melanconium* (*Sacchari ?*).

Fig. 23. $\frac{2}{1}$. Stylospores germinating after one day.

Fig. 24. $\frac{2}{1}$. Part of a mycelium developed in three days from the stylospore *c* in a hanging-drop of wine-must.

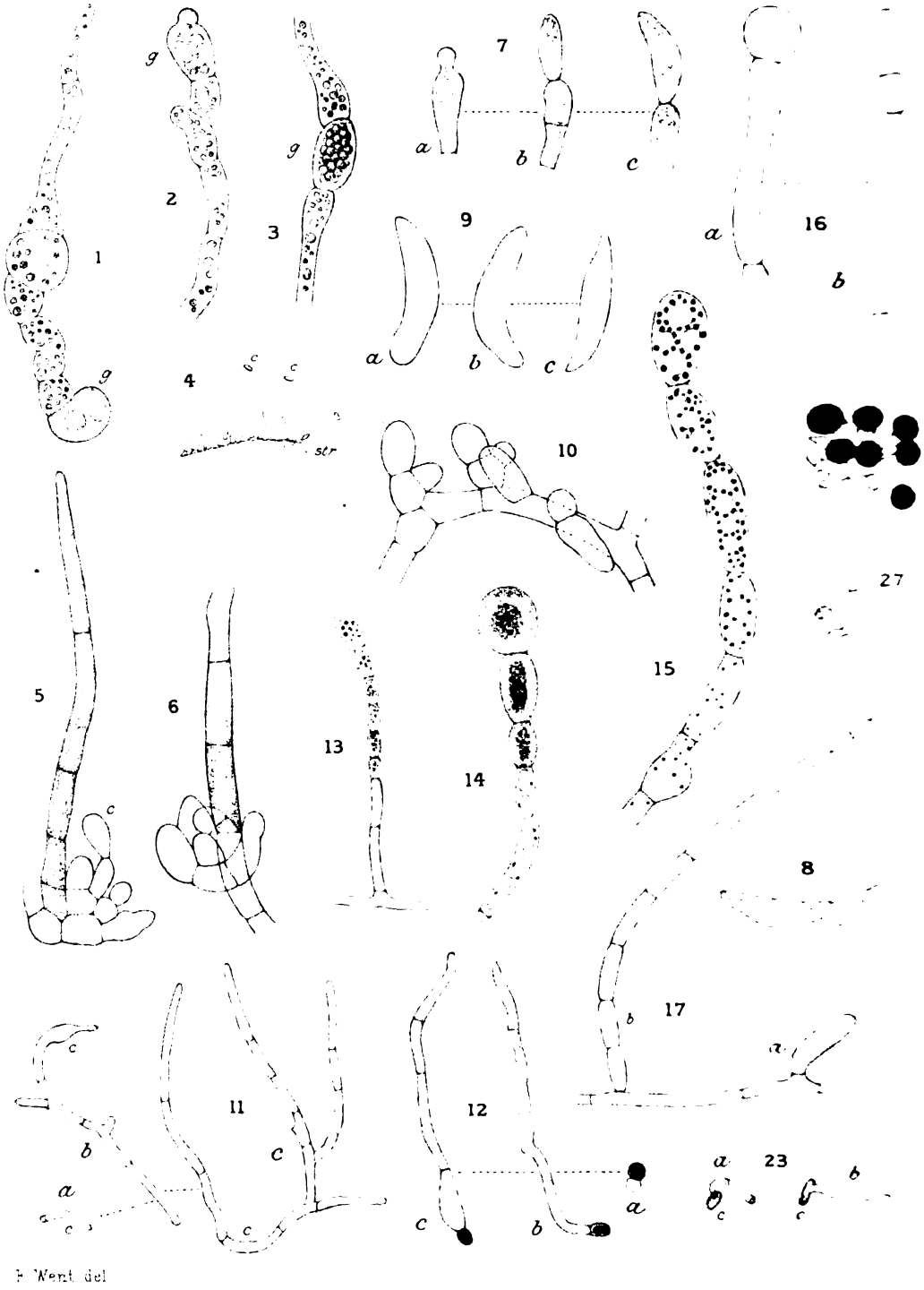
Fig. 25. $\frac{2}{1}$. The same as Fig. 24, but three days later.

Figs. 26, 27, 28. $\frac{2}{1}$. Mycelium with large black conidia.

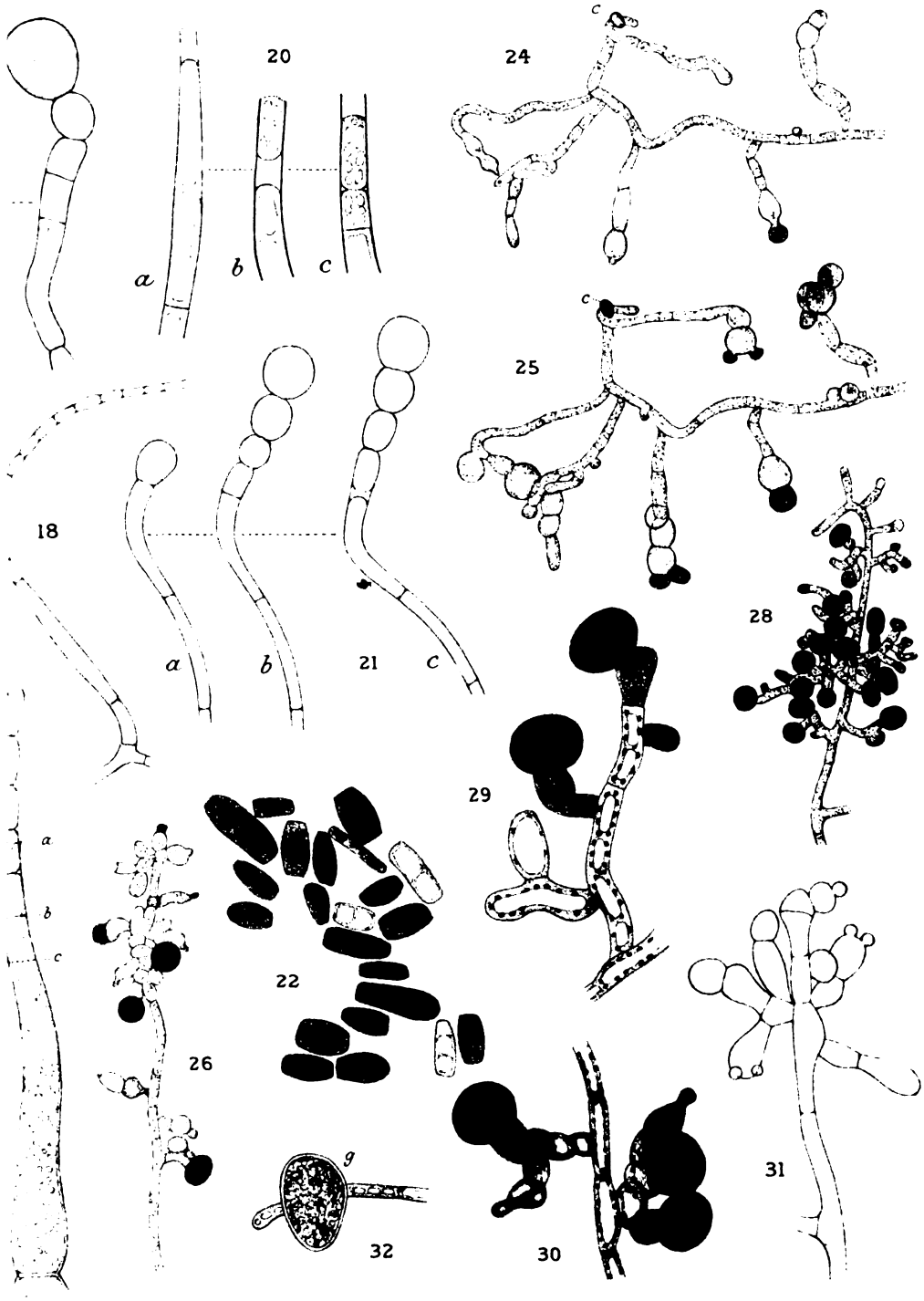
Figs. 29, 30. $\frac{2}{1}$. Mycelium with large black conidia.

Fig. 31. $\frac{2}{1}$. Mycelium with branches developing conidia.

Fig. 32. $\frac{2}{1}$. Mycelium with chlamydospore *g*.



WENT. — SUGAR-CANE DISEASES.



University Press, Oxford

The Anatomy of the Stem of Macrozamia compared with that of other genera of Cycadeae [1].

BY

W. C. WORSDELL.

With Plates XXVII and XXVIII.

COMPARATIVELY few investigations have hitherto been made into the minute anatomical structure of the stem of the Cycadeae; and any thorough and full account of this structure has been almost exclusively confined to two or three genera, as *Cycas*, *Encephalartos*, and *Stangeria*. Hence the examination of the stem of *Macrozamia*, a genus hitherto apparently untouched, seemed to promise some interesting results.

Historical Survey.

One of the oldest references to the internal structure of Cycadean stems is the investigation by Brongniart [2]; this work is consequently by no means free from erroneous interpretations. For example, the author entirely fails to recognize the presence of any phloem ('liber') in either of the two zones which he describes and figures in *Cycas revoluta*,

[1] From the Jodrell Laboratory, Royal Gardens, Kew.

[2] Recherches sur l'organisation des tiges des Cycadées, Ann. Sci. Nat. I, sér. XVI, 1829.

[Annals of Botany, Vol. X. No. XL. December, 1896.]

concluding that wood only is formed in this stem. His paper, however, is interesting and valuable, from the fact that he was the first to point out the real gymnospermous character of these stems and to refute the old idea of the similarity of their structure to that of Monocotyledons.

Von Mohl, in his paper 'Ueber den Bau des Cycadeen-stammes[1],' gives a considerable amount of detail about the structure of the stems of *Cycas* and two species of '*Zamia*' (*Z. latifolia* and *Z. horrida*). He states that in the pith of his *Zamia latifolia* there are a number of reticulately-united bundles, with scalariform or bordered pits, which pass singly through a medullary ray to the cortex. On the subject of anomalous thickening he says, speaking of *Cycas*, that some bundles from the normal ring pass out and run downwards through the cortex, arranging themselves side by side to form a second outer ring, of which, in '*Zamia*,' there are only very inconspicuous traces. It should be stated, however, that the plants called by him *Zamia*, are now placed by modern botanists under the genus *Encephalartos*[2].

In the monograph by Miquel[3] some of the anatomical characteristics of several genera are briefly touched upon, mention being made of such points as the nature of the pith, the pittings on the tracheides of the wood, &c.

In the work on the Cycads by Mettenius[4], which may perhaps be considered the best hitherto published, a great deal more light is thrown on the whole structure, and we are enabled to see very much more clearly how matters really stand with regard to the anatomy of these plants. It is from this paper that we have obtained most of our knowledge about Cycadean stems. He treats of the structure of *Cycas revoluta*,

[1] Abh. der k. b. Acad. zu München, I, 1832; republished and revised in Vermischte Schriften, 1845.

[2] See v. Mohl, loc. cit., Verm. Schriften, p. 198, foot-note. His '*Zamia latifolia*' was an African *Encephalartos*, and had nothing to do with the true *Z. latifolia*, Loddiges.

[3] Monographia Cycadearum, 1842.

[4] Beiträge zur Anatomie der Cycadeen, Abh. der K. Sächs. Gesellsch. der Wiss., VII, 1861.

Encephalartos horridus, *Dioon edule*, and *Zamia muricata*. In the two latter genera he finds no anomalous structure present.

Costantin and Morot[1], who investigated *Cycas siamensis*, with regard to the structure of the pericycle, state that it is in this tissue that the anomalous rings have their origin.

Count Solms-Laubach[2] has investigated the stem-structure of *Stangeria paradoxa*. The chief part of the paper is taken up with an elaborate description of the course of the ring of vascular bundles from the peduncle, which traverse the pith of the main stem for some distance before joining on to its vascular system. In this plant he finds no trace of any anomalous secondary thickening. The whole structure, with the exception of the medullary position of the cylinder from the peduncle, which is a phenomenon occurring also in other Cycads, is straightforward and normal.

Strasburger[3], in his work on the vascular tissues, gives an accurate account of our present knowledge of the anatomy of *Cycas*, but so far as the stem is concerned has not extended his investigations to other genera.

General Structure.

The chief peculiarity in Cycadean stems, which was recognized and described by former writers, is the presence of anomalous rings of secondary thickening outside the normal zone. This character, however, has hitherto been described for two genera only, viz. *Cycas* and *Encephalartos*; other genera, as *Zamia*, *Dioon*, and *Stangeria*, are said to be without this abnormality. The genus *Macrozamia* has apparently not yet been investigated.

Having had the opportunity of examining an old stem of *Macrozamia Fraseri*, Miq., of more than one foot in diameter, grown at Kew, I was able to determine that the same anomaly

[1] Bulletin de la Société Botanique de France, XXXII, 1885.
[2] 'Die Sprossfolge der *Stangeria* und der übrigen Cycadeen,' Bot. Zeitung, 1890.
[3] Hist. Beiträge, III, p. 151, 1891.

which occurs in the stems of *Cycas* and *Encephalartos* is also to be found in the stem of this plant, though somewhat less marked here than in the above two genera.

The following are the main facts of the general structure:—There is a large pith occupying half the diameter of the stem. Outside this is the normal vascular ring, consisting of about equal thicknesses of wood and bast. Immediately outside this is a similar ring of the same thickness, constituting the first anomalous zone. Immediately following this again is a third ring, consisting of very much smaller and more widely separated segments or bundles. This third ring is finally succeeded by a fourth series of segments, which, however, are developed only here and there, and for the greater part of the circumference of the stem are absent (Fig. 1). This structure is figured from a still larger stem (of *M. Moorei*, F. Muell.) in the Kew Museum.

This is what is usually seen in the lower region of the stem. Higher up, and especially near the apex, the third and fourth rings become more and more indistinct, and finally are no longer recognizable. The first anomalous ring alone persists as a conspicuous zone close to the apex of the stem.

I will now proceed to describe in detail the various tissues which go to make up the stem of this plant.

Structure of the Pith.

One of the most striking features of the whole structure, and one which is apparent at the first glance, is the presence in the pith of a dense network of vascular bundles, in fact the same phenomenon which occurs in the stem of *Encephalartos*, but which has been found to be absent in all other genera hitherto described.

If transverse sections are made in any portion of the pith, these bundles will usually be found in oblique or even in longitudinal section, but are scarcely ever cut through in an accurately transverse direction, showing that they do not run as isolated bundles perpendicularly through the tissue of the

pith; but that, on the contrary, their course lies in almost every direction, owing to the fact that they form part of an anastomosing system whose different branches traverse the length and breadth of the pith. If one of the main branches of this system is transversely cut through, it will be seen to be a bundle similar in size and appearance to one of the girdles of the cortex, consisting of a thick band of xylem and phloem produced by the activity of a cambium. Unlike the bundles of the cortex, however, its xylem and phloem have no regular orientation with regard to the vascular cylinder or the exterior of the stem, but lie, on the contrary, in any direction. The fact is that each branch of this medullary system, whether large or small, follows closely and is accompanied by a branch of a similar anastomosing network of mucilage-canals which traverse the pith in every conceivable direction. It is the mucilage-canal which invariably determines the orientation of the bundle, so that the phloem is always directed towards, and the xylem away from, the canal (Figs. 3, 6–8). It may frequently happen that a canal is accompanied by two bundles, one on either side, which anastomose with each other here and there on their course. But as the mucilage-canal twists and bends about through the pith, the bundle accompanying it appears first on one side of it and then on the other—seeing that it takes a short cut and does not follow every twist and turn of the canal—and in order, therefore, to preserve its orientation, it must necessarily undergo a twisting on its axis, the phloem being always on that side of the bundle nearest the canal (Fig. 3).

As showing how irregular and anomalous is the course of these medullary bundles, Fig. 5 represents a curious involution which very frequently takes place whereby the bundle or a portion of it assumes a perfectly circular course; it may also sometimes be seen entwining a mucilage-canal in this way. Renault[1], in his investigations of fossil plants, has figured similar contortions amongst the tracheides of the wood in the fossil genus *Cycadoxylon*.

[1] Structure comparée de quelques tiges de la Flore Carbonifère, Plate 14, 1879.

If transverse sections are made of the pith at the periphery, where it borders on the vascular zone, here and there one of the smaller branches of these medullary bundles may be seen running out, accompanied by its mucilage-canal, through a medullary ray between the segments of the wood. Its xylem and phloem join on in passing to that of the normal ring, while the mucilage-canal passes outward to the cortex to form a connexion with a similar system of secretory tissue in that part of the stem (Fig. 4).

In connexion with the xylem of the bundles, especially the larger ones, there is also very often to be seen what may be called a kind of transfusion-tissue, consisting of small, angular, isodiametric tracheides with bordered pits, running out alone into the tissue of the pith. These are usually connected with the first-formed elements of the bundles.

The proof that the medullary bundles of this plant, as in *Encephalartos*, form a separate cauline system of the stem, and are in no wise connected, as in *Stangeria* and others, with the conducting tissue of the peduncles, lies in their origin.

In transverse sections of the pith, taken from a region not far from the apex, mucilage-canals were seen in abundance, but no bundles whatever in connexion with them. A little further down, however, there began to be signs of a distinct differentiation amongst the cells immediately adjoining the mucilage-canal, and more especially on one side of the latter. The cells were much smaller and, therefore, more numerous. Still further down there appeared a difference in the cell-walls, especially in those of the outermost of these small cells, which gradually became thickened and lignified, these elements being evidently on the way to become tracheides; at the same time the innermost cells became further divided up as a preparation for phloem-formation. Eventually, the primary xylem and phloem became fully differentiated, each having respectively the orientation above described for the mature bundles (Figs. 6–8).

Anything of the nature of protoxylem is absent from these bundles, as one would naturally expect in secondary structures.

Structure of the Vascular Zones.

As seen in transverse section, the xylem of the normal ring is divided up, as in other Cycads already described, into narrow segments consisting of from one to three or four rows of elements in breadth, these being separated by medullary rays one or more cells broad. These segments of the xylem taper towards their inner margin where they abut on the pith. They appear usually to be arranged in groups with wider medullary rays separating such groups. As the different parts of the xylem, as well as of the phloem, are not all cut through in an equally accurate transverse direction, but are, on the contrary, often very obliquely sectioned, some of the vascular segments must depart considerably from the normal vertical course, and this was seen in longitudinal sections to be the case in nearly every part of the stem.

The phloem, in transverse section, appears to be composed of fairly regular rows, 2–4 cells broad, of sclerenchymatous elements, and of thin-walled small-celled tissue, the whole being divided up into segments, as is the xylem, by the medullary rays. On the outer side are seen the crushed remains of the primary phloem. On the inner side the cambium, forming a conspicuous zone, separates the secondary phloem from the xylem. Large idioblasts or stone-cells occur everywhere scattered about amongst the cells of the rows and in the medullary rays.

The xylem and phloem are of almost equal thickness. They are moreover, when observed under a low power, by no means easy to distinguish the one from the other, the lignified fibrous elements of the phloem being readily mistaken for tracheides.

A radial longitudinal section shows the wood to be of the usual Cycadean character; and owing to the tangentially-curved course of the tracheidal bands, the appearance is given, in most sections, of alternating zones of tracheides and ray-parenchyma. The protoxylem has completely disappeared

from the vertical strands of wood owing to its obliteration by the expanding parenchyma of the pith. It is only where the xylem of a leaf-trace is curving outwards, as a broad band of tracheides, that the typical spiral and reticulate elements are preserved; as they are in this position, perhaps, somewhat better protected against tension than elsewhere, though even here they are mostly isolated and broken up into fragments.

On the interior vertical face of the wood, where the protoxylem would naturally be sought, are often to be found the peculiar, irregular tracheides, resembling transfusion-tissue, above described as accompanying the medullary bundles.

The tracheides composing the secondary wood have the normal bordered pits on the radial walls. On the tangential walls very minute simple pittings were frequently observed.

Here and there, between the tracheides, are rows, usually one cell in thickness, of parenchyma, but there is only a comparatively small quantity of this present in the wood.

A characteristic feature of the radial section of the wood is the large number of out-bending strands of tracheides which, passing through the medullary rays, are continuous with the girdle leaf-traces of the cortex.

In a radial section of the phloem the greatly-elongated fibres are the most prominent objects. They serve, doubtless, to add strength and durability to a stem so largely built up of parenchymatous tissues. Side by side with these are seen the sieve-tubes, much elongated elements on whose obliquely-extended terminal and lateral walls very numerous sieve-plates of diverse shapes are seen, even without any staining. No callus, however, could be detected after repeated attempts at staining with aniline-blue. This was also the result of Strasburger's[1] investigation in the case of *Cycas*, who states that the cambial activity having ceased in this vascular ring, the sieve-tubes have lost their function, and therefore no longer deposit callus on their plates. But

[1] Loc. cit., p. 154.

the fact that in none of the rings could I detect any callus-formation seems to point to the conclusion that the growth of the stem as a whole had been arrested; this seems the more probable from the fact that the whole organ was infested in every part, from apex to base, with the mycelium of a Fungus, a condition of things which might tend to materially affect the normal functions of the stem. Indeed, the reason why this stem had been handed over to the Laboratory was that the apex was in a state of decay, and the plant had therefore become worthless for purposes of cultivation. Bordering on the sieve-tubes are extremely narrow long elements which are albuminous cells. Ordinary phloem-parenchyma, consisting of broader, shorter cells, is also present.

Returning once more to the transverse section from the lower part of an adult stem, we find, immediately abutting on the normal vascular ring, a second ring equal, or almost so, in breadth to the first. The inner portion of its wood abuts directly on the outer, first-formed phloem of the normal ring (Fig. 2).

It may be mentioned here that the segments of each of these two successive rings do not always lie evenly parallel one with the other. Occasionally one sees a large, wedge-shaped segment, really appertaining to the second ring, which has become pushed out of line, and lies embedded in a large medullary ray of the normal ring; so that it is at first not quite easy to tell to which of the two rings it really belongs (Fig. 1). There is, indeed, especially in the lower region of the stem, great irregularity in the whole structure, which may be partly due to the free play afforded to the expansion of the parenchymatous tissues in a stem whose vascular portion is so loosely compacted.

This first anomalous ring succeeds the normal ring just as has been described for *Cycas* and *Encephalartos*; and, at first, one would suppose that, in the plant I am describing, there was no more to be added to this point. But, on a subsequent more minute examination of the region where the phloem of

the normal ring abuts on the innermost tracheides of the first anomalous ring, an additional anomaly was perceived, which, in previous examinations of similar structures, had been entirely passed over. Here, between the two rings, a tertiary cambium had arisen, whose activity had produced a single small and isolated bundle, the peculiarity of which, however, lay in the fact that the orientation of its parts was reversed, its phloem being directed towards that of the normal ring and its xylem towards that of the anomalous ring (Fig. 9 *avb*^{3}).

Owing to the expansion of the phloem-parenchyma of the normal ring, some of the phloem-elements, such as the fibres, were seen lying outside the little bundle, which latter had thus become partially embedded in the phloem, and also, by the pressure of the parenchyma, rather distorted and shapeless. In sections of the same region from other parts of the stem a similar small bundle was observed, but with a different orientation, as it lay sideways, the plane of symmetry of its collateral structure forming a right angle with that of the two rings. In another case it was observed to lie, with the normal orientation, in a medullary ray at the side of the segments, but still in the region between the two rings. But I look upon these last two positions as departures from the normal orientation of the bundle, being irregularities due, probably, to the pressure of the surrounding parenchyma; much the more usual position is the inverted one above described. In other parts of the stem this cambium was seen to be less active than in the cases just described, and to form fewer elements of xylem and phloem, so that the tiny bundles thus formed are scarcely perceptible. Again, where a segment of the first anomalous ring is but feebly developed and but very few xylem- and phloem-elements are formed (for in certain parts of the stem this anomalous zone may be broken up into comparatively small segments), immediately on its inside, only separated from it by a few large parenchymatous cells, is frequently seen a minute bundle, or rather a half-bundle, consisting of two or three radial rows of phloem-

elements, of which the innermost one or two are seen to be lignified and represent fibres. This probably represents the same formation as the inverted small bundle above described, although, in this case, no xylem has been added by the cambium.

If we now pass outward beyond the first anomalous ring, on its immediate outer limit a third zone is met with, composed of bundles or segments, as above stated, which are very much smaller in size and less closely and compactly arranged than those of the first two rings. This zone constitutes the second definite anomalous ring produced by an extra-fascicular cambium. The segments composing it have the usual wedge-shaped contour, and in many parts of the stem are very irregularly arranged. They often leave the vertical and run in a tangential direction. These remarks apply to the lower region of the stem; in the upper portion no second zone of anomalous thickening is usually distinguishable, and the first ring, as also the normal vascular zone, is much less strongly developed.

In conjunction with the second anomalous ring the same phenomenon of inverted cambial activity occurs, but here in a more easily distinguishable and clearly defined manner. On the inner side of several of the segments, and separated from them by a few layers of parenchyma, appeared a bundle or segment, usually of smaller size, with inverted orientation, so that its xylem was placed directly opposite that of the normally orientated segment in some cases, while in others the bundle lay obliquely and at an angle with the other (Figs. 2, 10). The elements composing these inverted segments are not always so well developed and their walls not so sharply defined as those of the main segments, facts which tend to make them insignificant in appearance and easily passed over. Such a bundle was sometimes represented by only a mere rudiment made up of a few elements. In one or two cases, where the inverted segment lay rather obliquely, it formed an almost continuous zone with the normally orientated segment, thus indicating a tendency towards the formation of a concentric

bundle. It is worth noting that here and there in the space between an inverted and a normally orientated segment, several of the short, angular tracheides with bordered pits above described as occurring in conjunction with the medullary bundles and the xylem of the principal zone, were seen (Fig. 10); the presence of these added to the remarkable resemblance which the whole structure bore to the *concentric cauline bundles* in the cortex of *Cycas*, for here also these curious tracheides occur in the so-called pith. Where fragments of a third anomalous ring occur in the shape of a few scattered bundles, these may also be accompanied by a similar inverted bundle.

This peculiar tertiary cambial formation in connexion with the anomalous zones of secondary thickening in the stem of this plant has not hitherto been observed in the stem of any other genus. I could myself find no trace of a similar structure in connexion with any of the anomalous rings of *Cycas media*, of which plant I examined a very large stem with about a dozen vascular zones. At the same time, I believe, from a comparison made between the two, that this structure is homologous with that of the concentric cauline 'bundles' so well known to occur in the cortex of *Cycas*. These latter appeared, in the adult stem which I examined, as tangentially-extended zones, each of which had an orientation, as regards its xylem and phloem, the reverse of that in the other, the central region being filled with the short tracheides already mentioned.

This anomaly in *Macrozamia* I believe also to be similar in nature to, and homologous with, the well-known structure described by Gregg [1] in the roots of *Cycas Seemanni*.

The tranverse section of the stem shows clearly the passage of the leaf-trace bundles through the broad medullary rays from the cortex, traversing the successive vascular rings, to the normal cylinder. They may have connexions during their course with segments of each of the rings, thus establishing

[1] Anomalous Thickening in the Roots of *Cycas Seemanni*, Ann. of Bot., Vol. i, 1887.

between the vascular zones and the leaves a direct system of communication.

The direct connexion existing between the several rings of vascular tissue, apart from that afforded by the leaf-traces, was very clearly seen by the aid of radial sections which exhibited at intervals, especially between the normal and the first anomalous rings, very broad bands of wood and bast connecting the two both in a downward (the more marked of the two) and in an upward direction. Connexions in both directions may sometimes occur in close proximity, which thus give the curious looped appearance shown in Fig. 11, which is a diagrammatic, though accurate, representation of the structure. In this manner, by the continuity of the cambium of the different zones, a perfect communication is established between the various parts of the conducting-tissue of the stem.

It should be mentioned that the curious 'transfusion-tracheides,' which have already been several times referred to, constantly occur in considerable numbers (as is also the case in *Cycas media*) on the inner margin of the anomalous rings. They are of very various shapes, being either quite isodiametric, or oblong, contorted and shapeless, or more elongated and narrower, and having for the most part bordered pits on their walls. Whether they always formed a continuous series or not, as in normal transfusion-tissue, it was difficult to determine, and I did not succeed in so doing, but such is probably the case (Fig. 12 *t*).

A tangential section through the wood or the bast exhibits the curious looped structure caused by the bending of the tracheides and fibres in the tangential direction, this being due to the growth and expansion of the medullary rays which traverse these gaps. In each such loop or gap is seen a leaf-trace bundle with xylem directed upwards and one or two mucilage-canals (Fig. 13).

Structure of the Cortex.

The cortex occupies an area of the stem almost equal in thickness to that of the vascular zone. It is chiefly characterized

by the presence of a large number of leaf-trace bundles and, as in the pith, a network of mucilage-canals whose branches penetrate its tissue in every direction. These canals are often quite filled with cluster-crystals of calcium oxalate. Large stone-cells are of frequent occurrence throughout the cortex. The large number of bundles seen in transverse section of the stem on the inner margin of the cortex (Fig. 1 *g*) represent the inner ends of the girdle leaf-traces as they pass obliquely downwards to join the vascular zone. Their previous course through the cortex from their origin in the leaf-base is identical with that already well known in *Cycas* and other genera. On emerging from the leaf they pass for a long distance in the tangential direction, often two or three together, close beneath the surface of the stem, gradually, however, bending inwards and downwards towards the centre (Fig. 1 *g*). Some, however, follow a more direct route and bend off suddenly in a radial direction after a longer or shorter tangential course and pursue a direct path to the vascular zones (Fig. 16 *lt*). These latter, which do not occur in every region of the stem, may be associated together in considerable numbers, varying in size amongst themselves, being branching members of the original single bundle which passed into the stem from one side of the leaf-base. As they bend off in the radial direction they are often seen to arrange themselves in a curious arc, giving the appearance of an imperfect little cylinder of bundles, as seen in tangential section of the cortex. Some of the larger bundles, which run isolated through the cortex, show a distinct tendency towards a concentric structure. The leaf-trace girdles, during their tangential course around the stem, exhibit a rather striking structure from the fact that a large number of the tracheides of their secondary wood have become abnormally developed so as to resemble, in the spiral thickenings which they possess, the ordinary protoxylem. These tracheides are very broad elements, having more than twice the diameter of either the protoxylem-elements themselves or those of the rest of the secondary wood. They are, moreover, like those of

the protoxylem, more or less disorganized (Figs. 14, 15 *spx*^{2}). The normal part of the secondary wood only attains a comparatively small development, and its tracheides have the ordinary bordered pits (Figs. 14, 15 *nx*^{2}).

The explanation of the peculiar structure of these bundles lies in the fact that, from the direction of their course in the cortex, they would inevitably be submitted to an extreme tension in the tangential direction, so that an adaptation in a portion of the secondary wood, in the form of greater extensibility in the walls of the tracheides, became an imperative necessity, and thus we find the curious appearance in the secondary wood of tracheides with dense spiral thickenings. These bundles are orientated so that the xylem and phloem are directed towards the inside and the outside of the stem respectively. In many cases the outward course of the bundles was interrupted and cut short by the periderm. It is a fact worth noting that, as soon as the bundle leaves the tangential and assumes a more or less radial course, this abnormality in their secondary wood becomes very much less marked and tends to disappear altogether.

Uniting the various girdles in different regions of the cortex are 'radial connexions,' consisting of bundles equal in size to those of the girdles, and often fusing with them by means of a curious network of elements which are sometimes quite contorted and involuted.

On the outer limit of the cortex, the periderm forms a conspicuous layer, recognizable with the naked eye as a narrow white zone with an extremely irregular, sinuous course. It consists, on its outer side, of a layer of crushed cork-cells, and on its inner side, of a thick zone of phelloderm, built up of large square or variously-angled idioblasts or stone-cells, alternating with much smaller, thin-walled, colourless cells. The cells of this zone were seen to be arranged in radial rows continuous with those of the cork-layer on the outside. The periderm by successive layers cuts off, firstly, bit by bit of the leaf-bases, as shown in Fig. 1, where the dark lines (*pd*) represent this tissue, the deeply-shaded portions

between them being the dead, brown, parenchymatous tissue of the leaf-base, in the still living portion of which latter the leaf-traces are seen passing inwards to the cortex. After the leaf-bases have been disposed of, the periderm continues to eat its way into the cortex. Its successive layers arise in a very irregular manner, and often in the phelloderm of the older one (Fig. 17). There is no clean excision of the tissues along a definite line, as in *Zamia*, whereby a smooth even bark is obtained; but the outer surface of the stem, owing to this irregular sinuous formation of the periderm, is left very rough and jagged, consisting either of the remains of the leaf-bases or of dead portions of the cortex.

Owing to the periderm-formation it is difficult, in an old stem, always to obtain a proper clue as to the course of many of the leaf-traces in the extreme outer part of the cortex, as this region is often entirely cut away.

Summary.

The most noteworthy characters exhibited by the anatomical structure of the stem of *Macrozamia* are the following:—

1. The well-developed *medullary system of bundles*, which has a distinct adventitious origin of its own and is not directly continuous with the primary leaf-trace system, but only forms secondary anastomoses with it, being connected, by means of the medullary rays, with the various parts of the vascular ring. The curious contortions and involutions which some of the bundles of the pith undergo are also to be noted.

2. The *anomalous zones of secondary thickening* which, to the number of two or three, surround the normal cylinder. Of these the innermost is the best developed, being as thick as the normal ring upon which it immediately borders, and is the only one quite readily distinguishable with the naked eye on the cut surface of a stem. The second and third rings are much more feebly developed, and, in the upper part of the stem, often entirely absent. They are usually composed

of small, scattered segments, consisting of equal parts of xylem and phloem, and either abutting directly on the phloem of the first anomalous ring, or separated therefrom by a few layers of parenchyma.

3. The presence of a *tertiary cambium* which, arising in isolated places in the parenchyma either between the normal and the first anomalous ring, or between two anomalous rings, forms wood and bast with inverted orientation, such that its xylem is usually directly opposite that of one of the segments of an anomalous ring. This orientation of the bundles, especially in those near the normal ring, is, however, subject to the variations described in the foregoing pages, as is also the amount of tissue formed by this cambium.

4. The irregularity and displacements of many portions of the vascular tissue, owing to the great expansion of the parenchyma and the medullary rays between the various segments composing the rings.

The occurrence of the anomalous zones of thickening and of a tertiary cambium producing bundles with inverted orientation in this stem, can scarcely be passed over with a bare statement of the facts. I have been much struck with the appearance of these structures, especially after comparing them with anomalies in the stem of *Cycas*, and have therefore deemed it worth while to put forward a few considerations with regard to them, before drawing this paper to a close.

It appears to my mind highly probable that these structures have a phylogenetic and not a merely physiological significance. I would emphasize the great similarity existing between the structure, such as that represented in Fig. 12, and that of a concentric cauline bundle of *Cycas*. There are the same 'transfusion-tracheides' between the two oppositely-orientated strands of vascular tissue, a fact which seems to show that the parenchyma separating the two strands in *Macrozamia* is identical with the so-called pith of the cortical bundles in *Cycas*.

The whole structure, both of the anomalous zones and the tertiary cambiums, recalls strongly that of the stem of the

Medullosae [1], a fossil group with many Cycadean affinities; but it would be rash and premature to suggest here a homology between the two.

From these considerations I have been led to give the following provisional suggestion, viz.—

That the anomalous structures in Cycadean stems, and especially those in the stem of *Macrozamia*, are remnants of some ancient structure once common to a large group of plants. That this structure consisted of rings or layers of concentric vascular strands. That, as time went on, and greater specialization in the conducting-tissues arose, and a need for the formation of a larger amount of this tissue became urgent, the cambium of the inner portion of each such concentric strand gradually became less and less functional, that of the outer portion, on the contrary, more and more active, so that a much larger quantity of wood and bast became formed on the outer side of each strand than on the inner side, for this was the surest and best means of economizing both space and expenditure in the building up of an efficient conducting tissue for the stem.

The result is, finally, the structure, as we at present know it, in the stem of *Cycas*, *Encephalartos*, and *Macrozamia*. Some evidence for its origin lies in the relics of the inner cambium of the concentric strands in the vascular zones of *Macrozamia*; in the 'transfusion-tracheides' constantly found on the inner margin of every ring both of *Cycas* and *Macrozamia*; and in the presence of rings of concentric bundles, still preserving the old structure, in the cortex of *Cycas*.

5. The presence of leaf-traces in the cortex which run directly inward to the vascular rings after a very short tangential course, without describing the curves characteristic of the girdles in this and other Cycads.

6. The structure of the girdles during their tangential

[1] Göppert u. Stenzel. 'Die Medulloseae, eine neue Gruppe der fossilen Cycadeen,' Palaeontographia, Vol. 28, 1881. Sterzel u. Weber, 'Beiträge zur Kenntniss der Medulloseae,' XIII. Bericht der Naturwissenschaftlichen Gesellschaft zu Chemnitz, 1893–1896.

course, in which a large number of the tracheides of the secondary wood become spirally thickened, thus participating in the function of the protoxylem.

7. The structure and mode of action of the periderm at the periphery of the cortex, of which the phelloderm is largely composed of stone-cells, and produces subsequently a second phellogen in its midst.

It will thus be seen that, although there are many characters which *Macrozamia* has in common with *Cycas* and other genera already described, this genus possesses characteristic anatomical features which up to this date have not been noticed in the literature dealing with other members of this important order.

In conclusion, I must express my thanks to Dr. D. H. Scott for the help and many valuable suggestions which he has given me.

EXPLANATION OF FIGURES IN PLATES XXVII AND XXVIII.

Illustrating Mr. Worsdell's paper on *Macrozamia*.

The following are the abbreviations used in the lettering of the figures: *p*, pith; *mb*, medullary bundle; *x*, xylem; *ph*, phloem; *nr*, normal ring; *nph*, normal phloem; ar^1, first anomalous ring; ar^2, second anomalous ring; vb^4, bundles formed by a fourth cambium; ax^1, xylem of first anomalous ring; avb^3, bundle formed by tertiary cambium; *mc*, mucilage-canal; *t*, 'transfusion-tracheides'; *mr*, medullary ray; *ct*, cortex; *g*, girdle leaf-trace; *lb*, leaf-base; *pd*, periderm; *c*, cork; *pdm*, phelloderm; *pgn*, phellogen; *px*, protoxylem; spx^3, spirally-thickened tracheides of the secondary wood; nx^3, normal tracheides of the secondary wood; *lt*, leaf-trace.

Fig. 1. Segment from a transverse section of a stem of *Macrozamia Moorei*, F. Muell., in the Kew Museum, showing the general arrangement of the tissues from the pith to the leaf-bases. Natural size.

Fig. 2. Transverse section of a portion of a similar stem of *M. Fraseri*, Miq., preserved in spirit, showing segments of the anomalous zones of secondary thickening. × 15.

Fig. 3. Medullary bundle following a mucilage-canal, showing especially well the orientation of the former in the different parts of its course. Diagrammatic.

Fig. 4. Transverse section of a portion of the periphery of the pith where a medullary bundle is in the act of passing out through the normal ring. × 30.

Fig. 5. An involuted portion of a medullary bundle. × 30.

Fig. 6–8. Transverse sections of incipient stages in the development of medullary bundles. Figs. 6 and 8, × 85; Fig. 7, × 45. For Fig. 8 see Pl. XXVIII.

Fig. 9. Transverse section of the region between the normal and the first anomalous ring, showing a small bundle with inverted orientation. × 45.

Fig. 10. Transverse section of a segment of the second anomalous ring, with a small inverted bundle on its inner side; 'transfusion-tracheides' between the two bundles. Slightly diagrammatic. × 30.

Fig. 11. Diagram of the normal and the first anomalous zone in longitudinal section, showing the oblique connexions between the two.

Fig. 12. Longitudinal section passing through the extreme outer limit of the normal zone and the extreme inner limit of the first anomalous zone, showing 'transfusion-tracheides' between the two.

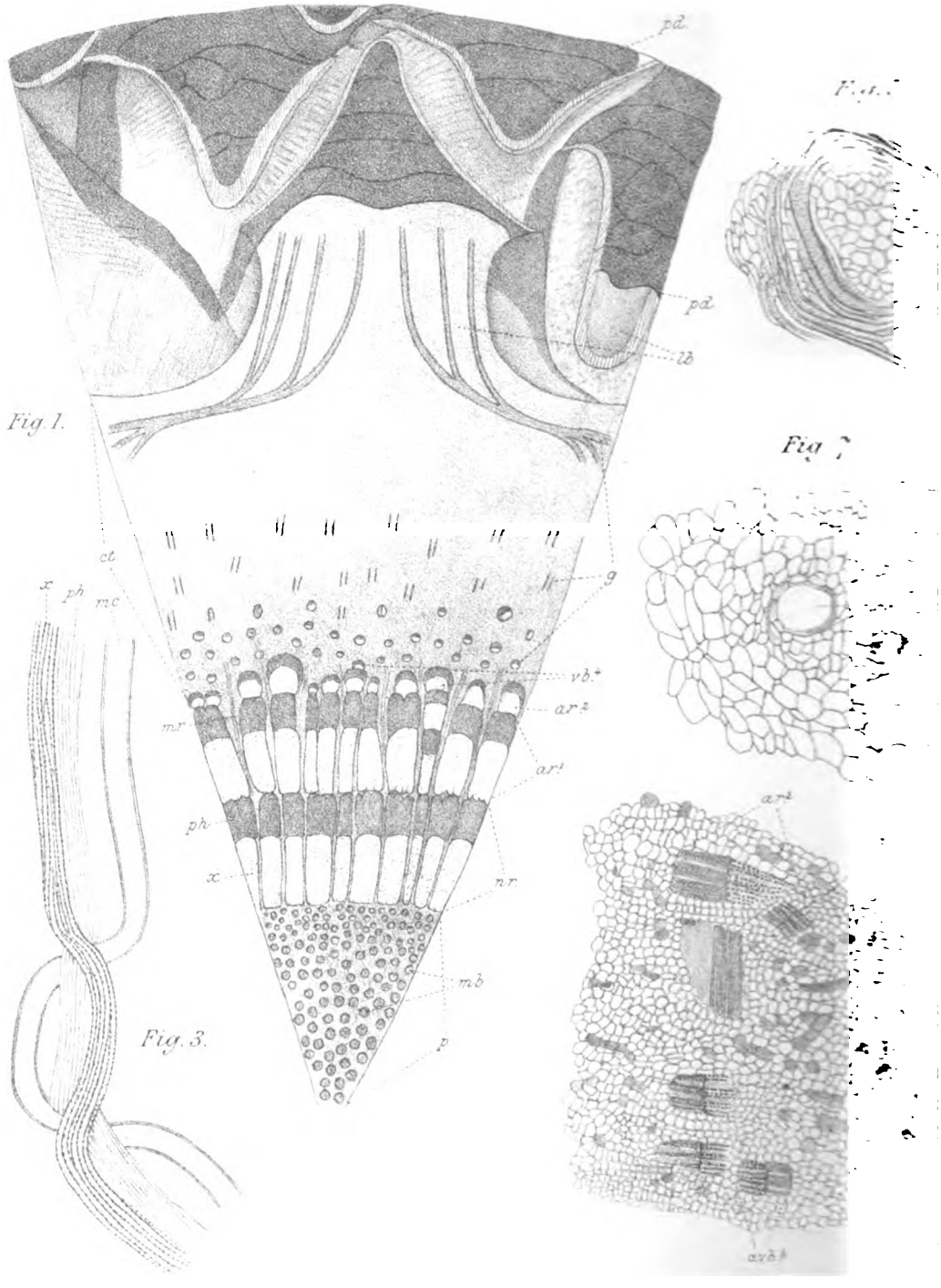
Fig. 13. Diagram of a tangential section of part of the xylem showing the local curved course of the tracheides owing to the expansion of the medullary rays. The leaf-traces in their outward course are seen in transverse section.

Fig. 14. Transverse section of a girdle leaf-trace during its tangential course through the cortex. × 60.

Fig. 15. Longitudinal section of the same. × 45.

Fig. 16. Diagram to illustrate the direct radial course of some of the leaf-trace bundles through the cortex. For this figure see Pl. XXVII.

Fig. 17. Section through an outermost portion of the cortex, showing the mode in which a new periderm-layer arises within the phelloderm of the old one. × 30.



W. C. W. del.

WORSDELL. — ON MACROZAMIA.

Fig. 4

Fig. 6.

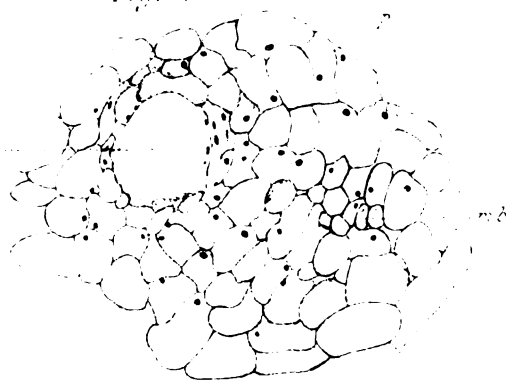


Fig. 16.

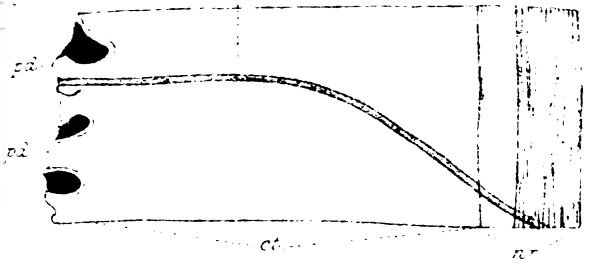
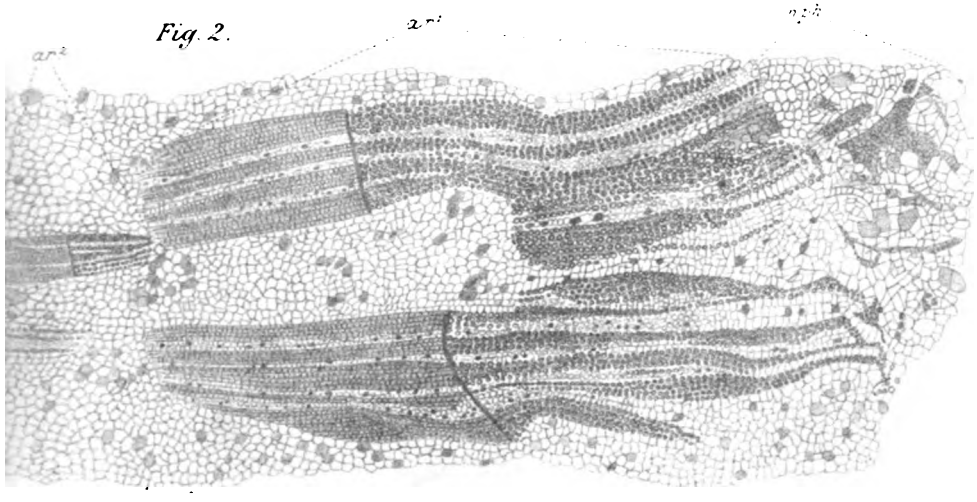


Fig. 2.



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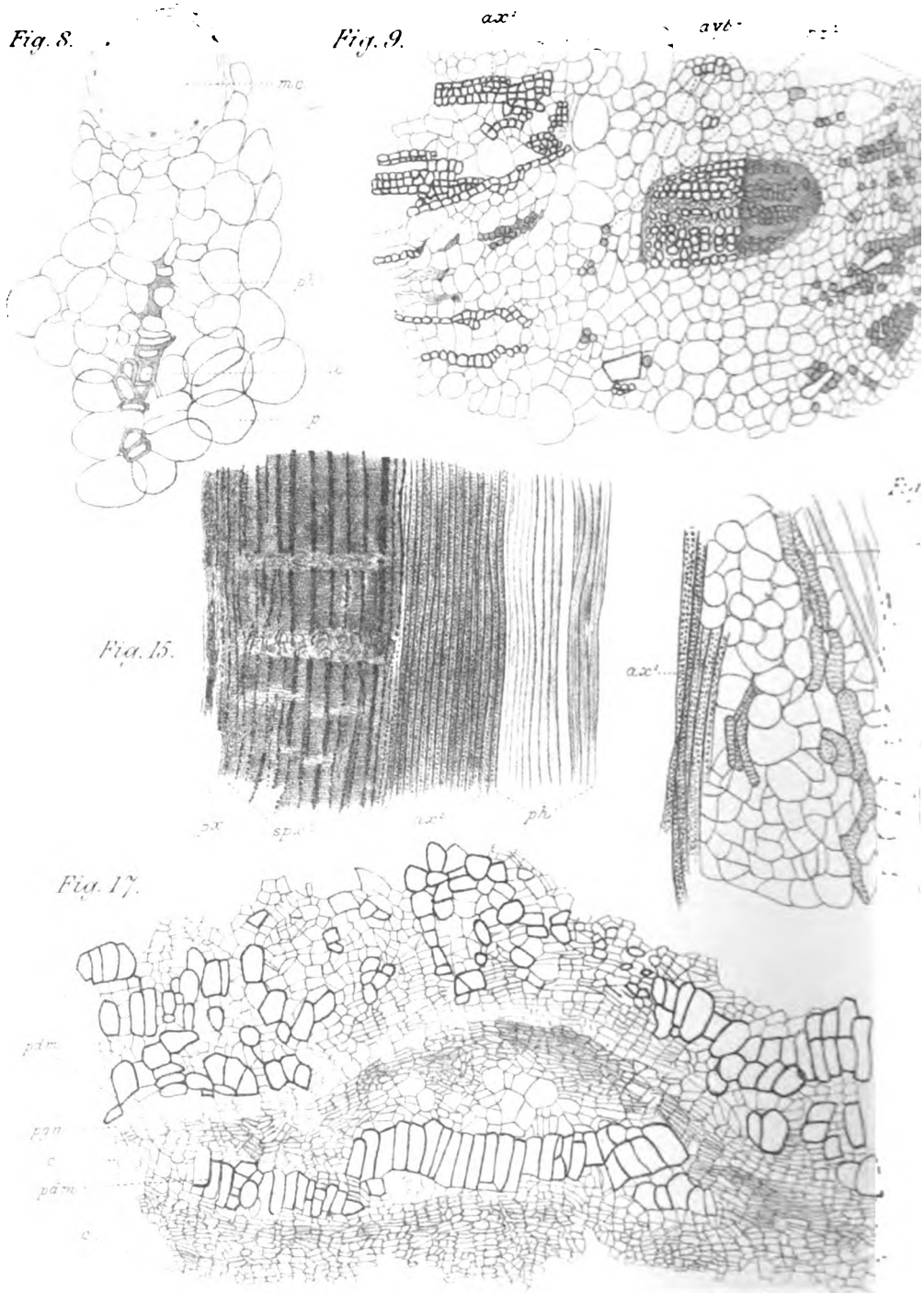


W. C. W. del.

WORSDELL. — ON MACROZAMIA.

Fig. 10.

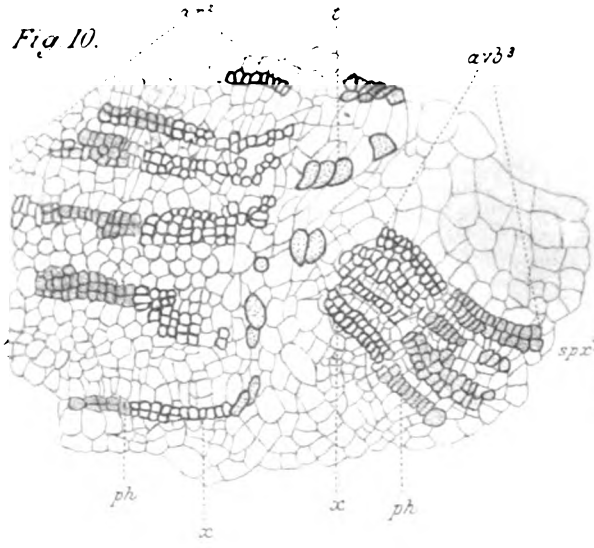


Fig. 14.

Fig. 13.

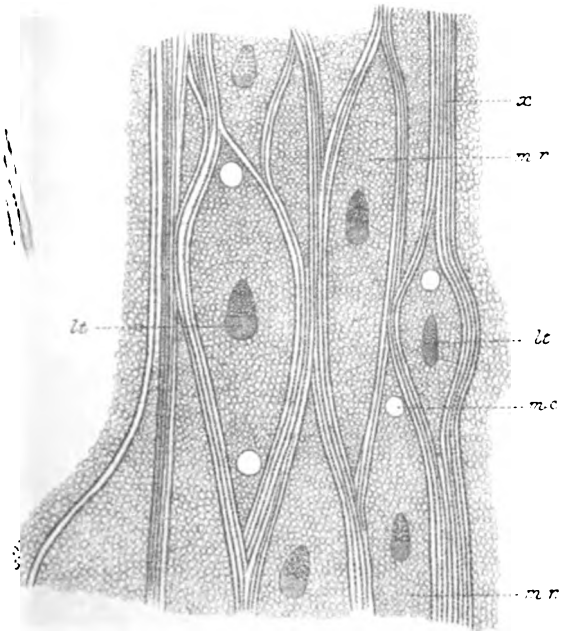
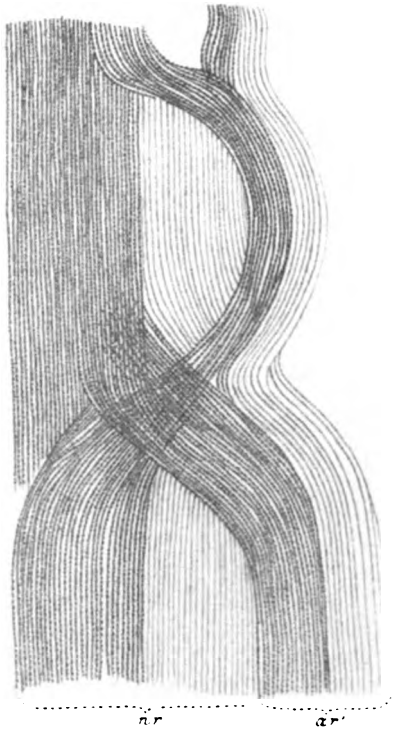


Fig. 11.



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NOTES.

THE OCCURRENCE OF A HYBRID GENTIAN IN BRITAIN.—On the celebrated Ridgeway, an ancient road which passes along the edge of the escarpment of the chalk range which looks over the vale of Berkshire, there are several British camps, one of the largest of which is Letcombe Castle, situated on the ridge to the south of Wantage. On this earthwork, and on the road which approaches it, *Gentiana germanica* occurs in considerable quantity and in excellent condition. This is in full flower in normal seasons in September. *G. Amarella* of English authors,=*G. axillaris* (Schmidt), Murbeck, also occurs there. It is usually in flower rather earlier than the former species, but in some seasons the time of flowering is more nearly conterminous. In 1892 I visited the place with Miss Beatrice Taylor, and found *G. germanica* in great abundance, and especially fine on the barer portion of the short turf. *G. Amarella* was more sparingly in flower, but there were some good specimens in the vallum. In this situation a few plants of a distinctly intermediate character were found. These had a longer and more conspicuous corolla than *G. Amarella*. The corolla was more cylindric than that of *G. germanica*, and the tint was nearer the dark purple of *Amarella* than the bluish purple of *germanica*. The pollen was defective, and the plant was in my opinion distinctly a hybrid of the two species with which it occurred. As the occurrence of a hybrid of these two species is only mentioned with a query in Focke's 'Pflanzenmischlinge,' I named it × *G. Pamplinii*, *G. Amarella* × *G. germanica* (after Mr. Pamplin, the reputed discoverer of *G. germanica* in Berkshire) in the Report of the Botanical Exchange Club of the British Isles, p. 379, 1892. Specimens were sent in that year to Dr. von Wettstein of

Prague for his opinion. At that time he was engaged on his Monograph of the genus *Euphrasia* (which excellent work is only recently published), so that he did not report on the specimen till this year, when he writes to me saying that he has no doubt from the intermediate characters, from the fact that the two supposed parents were growing together, and from the defective (58 per cent.) pollen, that the plant is a hybrid of the above plants, which he calls *G. Wettsteini*, Murbeck × *G. axillaris* (Schmidt). These names are synonymous with *G. germanica* and *G. Amarella* respectively. My friend, Mr. A. B. Jackson, has also found a hybrid of the two species at Watership chalk pit, on the north Hampshire chalk escarpment to the south of the Kennet, not far from Newbury.

G. CLARIDGE DRUCE.

Aug. 14.

THE HYBRIDS OF LINARIA REPENS AND L. VULGARIS IN BRITAIN.—The fact of the occurrence of hybrids of the two species above mentioned has been known for many years, but confirmatory evidence (if such were needed) of the strongest possible character has been afforded, during the last few years, in the neighbourhood of Oxford. In the counties of Oxford and Berks the distribution of *L. vulgaris* is fairly general, the plant being especially plentiful in light sandy soils. The occurrence of the second species is of a much more local character. Previous to the construction of railways, it was practically limited to the lower and upper chalk formations of the counties. The Great Western Railway, with its constant traffic, has much increased the area of its distribution, but until 1890 its northern limit may be said to have been at Didcot. On the soil which consists of bare chalk, *L. vulgaris* is practically absent, and there we may study *L. repens* in its natural condition. We shall find that it exhibits considerable variability in the colour of its flowers, which vary from nearly white to the darkest purple; it is rarely found of a coral pink tint; but the size of the flower, its shape, and the shape and direction of its spur, is fairly constant. When the soil is covered with a lighter subsoil, and *L. vulgaris* also grows with it, we shall then rarely search long without finding plants of an intermediate character. These plants are especially suitable for the purpose of observation, since, in the case of *L. vulgaris*, the large yellow flowers with an orange palate, and the much smaller flowers of *L. repens*, which are normally of a bluish or

purple tint and nearly always marked with striations (hence the name *L. striata*, which is one of its synonyms) of a deeper tint, offer such obvious characters for differentiation.

Up to the year 1890, only *L. vulgaris* occurred about Oxford; it then showed little variability either in its flowers or foliage. In that year a space of ground between the Great Western and the London and North-Western Railways was filled up with chalk rubble brought from the chalk district of Upton in Berkshire. With the chalk a considerable number of plants characteristic of that formation was introduced. In that year a profuse growth of *Iberis amara* and *Picris hieracioides*, and a less plentiful quantity of *Linaria repens*, *Campanula glomerata*, and *Bromus erectus*, covered the chalk with a very different vegetation from that which occupied the other side of the railway. In that year no hybrids of *L. repens* and *L. vulgaris* occurred; but the following year a very large number of hybrid plants occurred, in fact they were sufficiently numerous to attract the attention of Mr. B. S. Ogle to them as he was passing in a train. These hybrids can be roughly sorted into two groups, both of which are, however, much nearer to *L. repens* than to *L. vulgaris*, the influence of the latter plant being especially shown by the slightly larger size and the much paler (yellowish blue) tint, and by the more conspicuous orange palate. In 1892 the gap between the two species was practically filled up, but the plant with flowers nearly as large as those of *L. vulgaris* was quite rare, not more than eight or ten plants being seen. The influence of *L. repens* in this was practically limited to a very few pale striations on the corolla. The plant had narrower leaves than normal *L. vulgaris*, but the variation in the width of the leaves in that species is very considerable. To this I gave the name × *L. Baxterii* in the Report of the Botanical Exchange Club of the British Isles, 1893, p. 421. The commoner hybrid was one with nearly intermediate characters, but which was, on the whole, nearer to *L. repens*. This occurred by hundreds.

The year 1893 marked the maximum quantity of individual hybrids, when they considerably outnumbered the parent species. The chain of intermediates was again practically complete. Since that year the hybrids and *L. repens* have gradually diminished; this is owing to the competition of ranker weeds, which is assisted by the gradual covering up of the chalk with soil and refuse from the neighbourhood. It is probable that hybridization is assisted by the passing trains which

cause such a rush of air by the railway side where they grow. Seeds of plants which showed about a third of *L. vulgaris* and two-thirds of *L. repens* parentage when sown in light garden soil, yielded plants still nearer to *L. repens*; probably they were the result of crossing with the pollen of *L. repens*. Seeds of the second generation, however, yielded plants nearer to *L. vulgaris*; that is, they were about the same as the hybrid from which the first seeds had been collected. In this case pollen of *L. vulgaris* had been kept from them.

At Didcot the same series of hybrids has been noticed, but they occur in much smaller quantity.

G. CLARIDGE DRUCE.

Aug. 14.

THE ARRANGEMENT OF THE VASCULAR BUNDLES IN CERTAIN NYMPHAEACEAE[1].—One of the most remarkable characteristics of this order is the very extensive prevalence of the astelic system in the arrangement of the vascular bundles of their stems; however, during an examination into the structure of various members of the order, the fact that other systems of arrangement also are present came to light. Thus in *Nymphaea flava* and *N. tuberosa* the plants produce small tubers at the ends of stalks or stolons of greater or less length, and in these stalks or stolons the vascular bundles are not arranged in an astelic manner, but are grouped around three to five different centres, forming thus so many separate steles, or at least so many groups possessing all the characteristics of definite steles. Each of these is surrounded by its own endodermis, and is composed of three to four vascular bundles with very distinct and prominent phloems, while a small canal in the centre of the stele represents their disintegrated xylems.

The tubers formed at the end of these stolons bear buds which grow out into fresh rhizomes, the first internodes of which are very narrow and much elongated; in these, again, the vascular bundles (four to seven in number) exhibit a different arrangement, for they present none of the confusion found in the mature rhizome, but run perfectly longitudinally; either they all keep separate, or a varying number of them may be united to form pairs. When six of them are present and these are arranged in three pairs, the section presents

[1] Abstract of a paper read before Section K at the Liverpool Meeting of the British Association, 1896: see also Annals of Botany, Vol. x. p. 289.

a remarkable resemblance to that of the floral peduncle of *Cabomba aquatica.*

Again, in the rhizome itself the arrangement is not altogether astelic, for by the aggregation of the separated bundles of the stem a number of steles are formed, one in the region below the point of insertion of each leaf. These groups of bundles appear to be set apart for the especial purpose of bearing the adventitious roots, and they are to be found in varying degrees of perfection throughout the order. I found *Victoria regia* and certain species of *Nymphaea* to possess the most perfect root-bearing steles; they are composed of ten to twenty bundles arranged in a ring, and are perfectly distinct and well defined. On the other hand, in other species of *Nymphaea* and in *Nuphar*, the bundles set apart for bearing the adventitious roots are not arranged in a sufficiently regular manner to be considered as a stele, or are only laterally fused together to form an arc of greater or less extent.

D. T. GWYNNE-VAUGHAN.

CHANGES IN THE TENTACLE OF DROSERA ROTUNDIFOLIA, PRODUCED BY FEEDING WITH EGG-ALBUMEN[1]. —In unfed leaves fixed in watery picro-corrosive (sp. gr. 1·020) and stained with eosin-toluidin blue, the apical and lateral glands of the first or outer layer and also all the cells of the second or middle layer show a deep-blue cytoplasm, with nuclei possessing little chromatin proper, but large nucleoli and a granular nucleoplasm. Within one minute after feeding the blue cytoplasm becomes purple; after one hour it is greatly vacuolated and reddish purple; after twenty-four hours the blue material has disappeared, and only a few strands of a pink cytoplasm are to be seen. The nucleus after feeding loses the granular cytoplasm, the nuclear chromatin segments enlarge enormously, reminding one of the early stages of mitosis. The nucleolus has lost its red chromatin, and is not easy to see.

Recuperation of the cytoplasm is the result of nuclear activity, for the chromosomes enlarge during the period preceding the appearance of the granular nucleoplasm, which latter in every respect resembles the granular deposit of cytoplasm in immediate contact with the outer surface of the nuclear membrane. The cytoplasm is at first

[1] Abstract of a paper read before Section K at the Liverpool meeting of the British Association, 1896. For full account see Quarterly Journal of Microscopical Science, October, 1896.

purple in colour, but becomes blue after 6–7 days. After the 'secretion' of the cytoplasm the nuclear chromatin segments diminish in size, while the nucleoli become more and more evident, and the nucleoplasm has the same appearance as in a leaf which has never been fed. The third layer of gland-cells, perhaps concerned in the secretion of mucus, also shows marked changes; for the long spindle-shaped nuclei of the resting condition shorten within one minute, after ten minutes they are more or less globular, then pass through changes similar to those described above, and after some days resume their spindle shape—an indication of rest.

LILY H. HUIE.

A NEW CYCAD FROM THE ISLE OF PORTLAND[1].—Dr. Woodward lately obtained an exceedingly fine specimen of a cycadean stem from the Purbeck beds of Portland, which is now in the fossil-plant gallery of the British Museum. The stem, which is probably the largest known, has a height of 1 m. 18·5 cm., and measures 1 m. 7 cm. in girth at the broadest part. A striking feature of the specimen is the conical apical bud enclosed by tapered bud-scales, bearing numerous ramental outgrowths on the exposed surface. The surface of the stem presents the appearance of a prominent reticulum of projecting ridges, of which the meshes were originally occupied by the persistent petiole-bases. The substance of the leaf-stalks has for the most part disappeared, while the interpetiolar ramental tissue has been mineralized and so preserved as a projecting framework. In structure the ramenta are practically identical with those of *Bennettites*, as described by Carruthers and other writers. The petiole-bases also agree very closely with those of *Bennettites*, consisting of a mass of parenchymatous tissue traversed by numerous vascular bundles and secretory canals, with a distinct band of cork at the periphery. No trace of any inflorescence has been found. It is proposed to name the plant *Cycadeoidea gigantea*.

A. C. SEWARD.

OBSERVATIONS ON THE LORANTHACEAE OF CEYLON[2]. **II. ANATOMICAL.**—*I. Emergences on the Embryo of Loranthus neelgherensis.*—The hypocotyl of the fully-developed embryo

[1] Abstract of a paper read before Section K at the Liverpool Meeting of the British Association, 1896.

[2] Ibid.

is densely covered with green columnar emergences, whose cortical cells contain chlorophyll, starch, tannin, and a substance giving the reactions of a fat. Irregular masses of a similarly reacting material are frequently found covering the cuticle.

A *single* stoma occurs on the free surface of each emergence, and in the embryo of this species stomata are confined to the emergences.

The cuticle covering the general epidermis is continuous over the guard-cells of each stoma, except for a small oval slit which allows of communication between the intercellular space and the air. The stomata thus suggest either a xerophytic habit for the plant or an abnormal function for themselves.

The emergences flourish during the germinating (epiphytic) stage, and later, when semi-parasitism is achieved, cease to be functional.

II. Mode of penetration into the host of L. neelgherensis.—Unlike many species, *L. neelgherensis* develops no well-marked organ of attachment (suctorial disc) at the free end of its hypocotyl.

Where much resistance to the entry of the sucker is offered by the host, there are formed at the edges of the attached surface of the hypocotyl a series of acropetally arising, hair-bearing cortical ridges. The later-formed ridges, wedging themselves in between the older ones and the bark, force these older ridges away. The firmly-attached hairs of each ridge so forced away tear off masses of the bark, and thus the softer tissues, through which the sucker readily and cleanly bores, are exposed by instalments. Where the sucker comes in contact with lignified structures, dissolution is more gradual, and stages of disintegration (erosion figures) are to be observed.

In *L. loniceroides*, where a well-marked suctorial disc is formed, attachment occurs once for all. This attachment is maintained (1) by the growth of the edge of the disc hard against the bark; (2) by the outgrowing hairs forming a matted sclerotic mass firmly fixed into the outer layers of the host.

F. W. KEEBLE.

PRELIMINARY NOTES ON FLORAL DEVIATIONS IN SOME SPECIES OF POLYGONUM[1].—The genus has long been known to show considerable departures from the arrangement and number of parts accepted as most typical (Per. 5. St. 5 + 3, C. $\frac{3}{5}$), such as is found in *P. Convolvulus*. Eichler's 'Blüthendiagramme,'

[1] Abstract of a paper read before Section K at the Liverpool meeting of the British Association, 1896.

for example, shows diagrams of several species as if characterized by constant differences of structures. Observation shows that in some species (e. g. *P. Convolvulus*) variations are comparatively infrequent and slight, but that in most (e. g. *P. Persicaria* and *aviculare*) they are extremely frequent, and lead to very great changes in floral structure. Often it is scarcely possible in such species to find two flowers alike on the same branch, or even on the same plant. Within a species individual plants show wide differences in the frequency and extent of variations.

A comparison of different species shows that while each varies, so as in the more variable species to cover almost the whole range observed in the genus, each shows a tendency to certain lines of variation. These tendencies are more alike usually in the more nearly allied species, so as to correspond in the main with the groups based on habit, and they lead from group to group.

The modes of variation commonly observed include almost all the recognized modes of departure from floral symmetry. They affect all the whorls. The *perianth* in some species is very constant. In others it habitually shows cohesion of two or more segments, or abortion in different degrees, or suppression of one or two (usually the inner) segments. Chorisis of a segment is less frequent. Enations from one or more segments are frequent in certain species, rare or absent in others. The *outer stamens* often show cohesion of the two in each pair, varying from the slightest union of the bases of the filaments to absolute union of even the anthers. Abortion (in all degrees to complete suppression) of one or more stamens is not rare, frequently reducing this whorl to $\bar{3}$ (less often to 2) in *aviculare*. Chorisis is not rare, especially of the unpaired stamen. The *inner stamens* seldom show cohesion (except in *aviculare* and its allies) with stamens of the outer whorl. Abortion (in all degrees to complete suppression) is very frequent, and in certain species (*amphibium*) this whorl has completely disappeared. In *aviculare* and allied species the inner whorl shows abortion less than the outer. Chorisis in the inner whorl most frequently shows itself in the posterior stamen. Adhesions of stamens to perianth segments and petalody of stamens are not frequent.

(In *P. amphibium*, the land form near Aberdeen very generally has the anthers very small or abortive, and the stamens hidden within the perianth, while the form growing in water has the anthers well

developed, and some or all exserted; neither form appears to seed habitually.)

The *pistil* in some species is very constant, while in others it shows all stages of cohesion and reduction to two carpels, this being the almost invariable number in certain species. Abortion is less frequent, and complete suppression cannot be distinguished from complete cohesion. Chorisis is very frequent in *aviculare* and some other species, in all degrees from a mere enlargement of one or more stigmas to an increase in number (up to seven), with corresponding modifications in structure in the ovary. Only one ovule has been observed in each ovary.

Markedly teratological forms have been met with, but are not included in this summary.

No very definite relation has been traced between the position of a flower on the axis and deviations in structure, though pressure tends to abortion or suppression of parts, especially of the sexual organs. (The flowers examined have chiefly been those sufficiently open to allow the natural arrangement to be noted without manipulation, to avoid displacement of parts, hence cleistogamous flowers are scarcely included.) The variability appears rather to express the result of an innate tendency to vary where not subject to the check of loss of fertility, the variations in *Polygonum* not leading to this loss.

The same number of parts in a whorl may be due to very different causes, and still more may the same number of stamens express very different arrangements in the flower; hence such a statement in a specific description as 'stamens usually six' is insufficient.

J. W. H. TRAIL.

REPORT

OF A DISCUSSION ON THE ASCENT OF WATER IN TREES

Held in Section K at the Meeting of the British Association, Liverpool, September 18, 1896.

MR. FRANCIS DARWIN read the following paper:—

WITHIN the last few years the problem of the ascent of water has entered on a new stage of existence. The researches which have led to this new development are of such weight and extent that they might alone occupy our time. It will be necessary therefore to avoid, as far as possible, going into ancient history. But it will conduce to clearness to recall some of the main stepping-stones in the progress of the subject.

The two questions to be considered are:—(1) What is the path of the ascending water? (2) What are the forces which produce the rise.

I. The first question has gone through curious vicissitudes. The majority of earlier writers assumed that the water travelled in the vessels. This was not, however, a uniform view. Caesalpinus, 1583, seems[1] to have thought that water moved by imbibition in the 'nerves.' Malpighi and Ray held that the vessels serve for air, and the wood-fibres for the ascent of water. Hales[2], who believed in the 'sap-vessels' as conduits, speculated on the passage upwards of water between the wood and the bark. Also[3], that water may travel as vapour, not in the liquid state. In the present century Treviranus[4], 1835, held that water travelled in vessels; De Candolle, 1832, that the intercellular spaces were the conduits. In Balfour's Manual of Botany, 1863, vessels, cells, and intercellular spaces are spoken of as transmitting the ascending water.

The change in botanical opinion was introduced by the great

[1] Sachs' Hist. of Bot. (English Trans.), p. 451.
[2] Vegetable Staticks, p. 130.
[3] Loc. cit., p. 19.
[4] Sachs' History.

[Annals of Botany, Vol. X. No. XL. December, 1896.]

authority of Sachs [1], who took up Unger's view [2] that the transpiration current travels in the thickness of the walls as water of imbibition.

Then followed the reaction against the imbibitionists—a reaction which has maintained its position up to the present time. Boehm, who had never adopted the imbibition theory, must have the credit of initiating this change; his style was confused and his argument marred by many faults, but the reaction should in fairness be considered as a conversion to his views, as far as the path of the travelling water is concerned. Nevertheless, it was the work of others who principally forced the change on botanists—e. g. von Höhnel [3], Elfving [4], Russow [5], R. Hartig [6], Vesque [7], Godlewski [8], and others.

II. The second question has a curious history, and one that is not particularly creditable to botanists generally. It has been characterized by loose reasoning, vagueness as to physical laws, and a general tendency to avoid the problem, and to scramble round it in a mist of *vis à tergo*, *capillarity*, *Jamin chains*, *osmosis*, and *barometric pressure*.

An exception to this accusation (to which I personally plead guilty) is to be found in Sachs' imbibition theory, in which, at any rate, the barometric errors were avoided, though it has difficulties of its own, as Elfving has pointed out.

But the most hopeful change in botanical speculation began with those naturalists who, concluding that no purely physical causes could account for the facts, invoked the help of the living elements in the wood. To Westermaier [9] and Godlewski [10] is due the credit of this notable advance, for whether future research uphold or destroy their conclusions, it claims our sympathy as a serious facing of the problem by an ingenious and rational hypothesis [11].

[1] Physiol. Végétale (French Trans.), 1868, p. 235, and more fully in the Lehrbuch. Sachs also partially entertained Quincke's well-known suggestion of movement of a film of water on the surface of vessels.

[2] Sitz. k.k. Akad. Wien, 1868. Dixon and Joly's paper in the Annals of Botany, September, 1895, gives evidence in favour of a certain amount of movement of the imbibed water.

[3] Pringsheim's Jahrb., xii, 1879.

[4] Bot. Zeitung, 1882.

[5] Bot. Centr., xiii, 1883.

[6] 'Ueber die Vertheilung,' &c., Untersuchungen aus dem Forst. Bot. Inst. zu München, ii and iii.

[7] Ann. Sc. Nat., xv. p. 5, 1883.

[8] Pringsheim's Jahrb., xv, 1884.

[9] Deutsch. Bot. Ges., Bd. i, 1883, p. 371.

[10] Pringsheim's Jahrb., xv, 1884.

[11] It is of interest to note that Hales, in speaking of the pressure which he found to exist in bleeding trees, says: 'This force is not from the root only, but must also proceed from some power in the stem and branches.' Veg. Staticks, 1727, p. 110.

We may pass over the cloud which arose to witness for and against these theories, and proceed at once to Strasburger's great work[1], in which, with wonderful courage and with the industry of genius, he set himself to work out the problem *de novo*, both anatomically and physiologically. In my opinion it is difficult to praise too highly this great effort of Strasburger's.

Strasburger's general conclusion is now well known. He convinced himself that liquid can be raised to heights greater than that of the barometric column, in cut stems, in which the living elements have been killed. Therefore, the cause of the rise could not be (1) barometric pressure, (2) nor root pressure, (3) nor could it be due to the action of the living elements of the wood. His conclusions may be stated as follows:—

(*a*) The ascent of water is not dependent on living elements, but is a purely physical phenomenon.

(*b*) None of the physical explanations hitherto made are sufficient to account for the facts.

Strasburger has been most unjustly depreciated, because his book ends in this confession of ignorance. I do not share such a view. I think to establish such distinct, though negative, conclusions would be, in this most nebulous of subjects, an advance of great value. Whether he has established these conclusions must of course be a matter of opinion. To discuss them both would be to go over 500 pages of Strasburger's book, and will not here be attempted. Conclusion (*a*) that the ascent is not dependent on living elements must, however briefly, be discussed, because it is here that the roads divide. If we agree with Strasburger, we know that we must seek along the physical line; if we differ from him, we are bound to seek for the missing evidence of the action of the living elements.

Schwendener's Criticism.—Perhaps the best plan will be to consider the most serious criticism that has been published of Strasburger's work, namely, Schwendener's paper 'Zur Kritik' &c.[2]

Schwendener objects that although a continuous column of water cannot be raised by air pressure to a greater height than that of the barometric column, yet when broken into a number of columns, as in the case of a Jamin chain, that a column considerably over 10 m., even as much as 13 or 14 m., of water can be suspended. This, though

[1] Leitungsbahnen, 1891.

[2] K. Preuss. Akad. 1892, p. 911.

not fatal to Strasburger's conclusions, is no doubt a serious criticism. For if 13 m. can be supported, some of Strasburger's experiments are inconclusive. He finds that a branch can suck up a poisonous fluid to over 10 m., and, as above explained, argues that all ascent above that height, not being due to barometric pressure or to the living elements (since the wood is poisoned), is for the present inexplicable. But, if Schwendener is right, the effect above 10 m. may have been due to atmospheric pressure. Askenasy (loc. cit. infra, 1895, p. 6) objects to Schwendener that the supposed action cannot be continuous. By repeating the diminution of air pressure at the upper end, the movement of water becomes less and less, and sinks to almost nothing. Askenasy adds, moreover, that the amount of water which could be raised according to Schwendener's theory would be very small.

One difficulty about Schwendener's theory is that the result depends on the length of the elements of which the chain is made up (such element being a water-column, *plus* an air-bubble). In his paper, 'Ueber das Saftsteigen[1],' he finds that the elements of the chain in *Fagus* equal in round numbers 0·5 mm. In his paper[2], 'Wasserbewegung in der Jamin'schen Kette,' he finds the element in *Acer pseudo-platanus*=0·9 mm., in *Acer platanoides* and *Ulmus effusa*=0·2. But the calculation (1892, p. 934) is based on the existence of a chain in which the water-columns are each 10 mm. in length, a condition of things which he allows does not occur in living trees.

But even if we allow Schwendener to prove theoretically the possibility of a Jamin chain being raised to a height much greater than that of a barometric column, I do not think he invalidates Strasburger's position. Schwendener's idea necessitates the travelling of a Jamin chain as a whole, i.e. the translation not only of water, but of air-bubbles. But this cannot (as Strasburger points out) apply to his experiments on Conifers, in which the movement of air to such an extent is impossible[3]. And for the case of dicotyledonous woods, Strasburger has shown that the movement of air is excluded by the fact that transverse walls occur in the vessels at comparatively short distances. In *Aristolochia* the sections may be as long as 3 m., but in ordinary woods according to Adler[4] we get: *Alnus* 6 cm.; *Corylus*,

[1] K. Preuss. Akad., 1886, p. 561.
[2] K. Preuss. Akad. Sitz., 1893, p. 842.
[3] 'Ueber das Saftsteigen,' Hist. Beiträge, v. 1893, p. 50.
[4] As quoted by Strasburger.

11 cm.; *Betula*, 12 cm.; *Quercus*, 57 cm.; *Robinia*, 69 cm. These facts seem impossible to reconcile with Schwendener's views.

Action of the Poisonous Fluids in Strasburger's Experiments.—The question whether the living elements are killed in Strasburger's experiments is of primary importance in the problem.

Schwendener does not criticize it at length; he seems to assume[1]—as far as I can understand—that since the death of the tissues extends gradually from the cut end upwards, there are living cells in the upper part which may still be effective. He also doubts 'whether the cells were always killed at once.' The first objection of Schwendener's may or may not be sound, but in any case it does not (as Strasburger points out) account for the experiment[2] in which an oak stem was poisoned by picric acid, and three days afterwards was placed in fuchsin-picric. The second reagent had to travel in tissues already killed with picric acid, yet a height of 22 m. was reached.

The question whether the reagents kill the cells in Strasburger's experiments does not lend itself to discussion. It is difficult to see how they should escape, and we have Strasburger's direct statement that the living tissues were visibly killed. It must not be forgotten that in some of his experiments the death of the tissues was produced by prolonged boiling, not by poisons[3]. Thus the lower 12 m. of a *Wistaria* stem were killed in this way, yet liquid was sucked up to a height of 108 cm. In the Histolog. Beitr. v. p. 64, he has repeated his air-pump experiment, using a boiled Yew branch, and found that eosin was sucked up from a vessel in which almost complete vacuum was established, so that the action of living elements and of atmospheric pressure was excluded.

On the whole, the balance of evidence is, in my judgment, against the belief that the living elements are necessary for the rise of water. In other words, I think we should be justified, from Strasburger's work, in seeking the cause of ascent in the action of purely physical laws.

Strasburger's general argument from the structure of wood.—It seems sometimes to be forgotten that, apart from the physiological or experimental evidence, there is another line of argument founded on the structure of wood. Strasburger's unrivalled knowledge allows him to

[1] Zur Kritik, loc. cit., 1892, p. 935.
[2] Hist. Beitr. v. p. 12.
[3] Leitungsbahnen, p. 646.

use this argument with authority, and he seems to me to use it with effect. Thus[1] he points out that though in coniferous wood the action of the living elements in pumping water is conceivable, yet this is far from being universally the case. He points out that in the Monocotyledons such theories meet with almost unconquerable difficulties. This is, he says, especially the case in *Dracaena*. He goes on to point to difficulties in the case of such Dicotyledons as *Albizzia*. The case may perhaps best be put in the generalized manner that Strasburger himself employs[2]. If the living elements are of such importance as Godlewski, Westermaier, and Schwendener hold, we ought not to find these difficulties; we ought rather to find structural peculiarities pointing distinctly to the existence of such functions. For instance, we ought to find the tracheal water-path actually interrupted by living elements, which might act like a series of pumping stations one above the other. It should, however, be remembered that if we deny the importance of the medullary rays and other living elements in raising water, we ought to be able to point more clearly than we can at present to the function of the medullary rays and to structural adaptations to these functions.

The work of Dixon and Joly and that of Askenasy.—I now pass on to recent papers in which Strasburger's indications to search along a purely physical line have been followed. In 1894 Messrs. Dixon and Joly[3] first enunciated an entirely new theory, depending upon the quality which water possesses of resisting tensile stress. To most botanists the existence of this quality is a new idea. To believe that columns of water should hang in the tracheals like solid bodies, and should, like them, transmit downwards the pull exerted on them at their upper ends by the transpiring leaves, is to some of us equivalent to believing in ropes of sand. Meanwhile Askenasy had independently hit on a similar theory which was published[4] after the appearance of Messrs. Dixon and Joly's research.

Askenasy has earned the gratitude of his botanical readers by giving some of the evidence which demonstrates the existence of this property of water[5]. A tube a metre in length was filled by Donny with water,

[1] Hist. Beitr. v. p. 17. [2] Loc. cit., p. 20.

[3] Proc. Roy. Soc., Vol. lvii, No. 340 (1894). Also Annals of Bot., Vol. viii; Phil. Trans., Vol. 186, 1895 (B).

[4] Verhand. a. d. naturhist. med. Vereins Heidelberg, N. F., Bd. v, 1895; and N. F., Bd. v, 1896.

[5] He gives references to Donny, Poggendorff's Annalen, 67. Bd. (143. Bd. d. g. R.)

and the remaining space was as far as possible freed from air. When the tube was placed vertically, the water-column at the upper end hung there, and could not be made to break or free itself from the glass by violent shaking. Berthelot filled a thick-wall capillary tube completely with water at 28°–30° C.; it was allowed to cool to 18°, so that the space left by the shrinking of water was filled with air. It was then sealed up and again warmed to 28°–30°, so that the air was dissolved in the water. When it was allowed to cool again it retained its volume, filling the tube completely. A slight shake, however, allowed the water to break and return to its proper volume at 18° with the appearance of a bubble of air. In this experiment the water contained air, yet it seems to have been until recently assumed by some physicists that to show cohesion, water must be air-free. If this were the case, the application of the principle to plants would be impossible. Dixon and Joly have, however, proved that this is not so, and this forms an important part of their contribution to the subject.

They also [1] investigated the amount of tension which water under these circumstances will bear, and found it about equal to seven atmospheres. If, therefore, the leaves at the top of a tall tree can exert the requisite upward pull on the water in the trunk, it seems certain (if no other conditions in the problem interfere) that the pull can be transmitted to the level of the ground. This opens up the question whether the leaves can exert this traction on the water in the tracheals, and what is equally important, Are there any factors in the problem incompatible with the theory?

1. *The sucking force of the leaves.*—In Dixon and Joly's first paper [2] they assume that tractional force is given by the meniscuses 'formed in the membranous *réseau* of the evaporating cell-walls,' as well as possibly by the osmotic action of the cells of the mesophyll. We shall take these theories in order. Our knowledge of the cell-wall does not allow us to believe in the existence of pores visible with even the highest powers of the microscope. Dixon's more general expression [3], 'surface tension forces developed in the substance of the

1846, p. 562; Berthelot, Annales de Chimie et de Physique, S. 3, t. 30, 1850, p. 232; Worthington, Proc. Roy. Soc., Vol. l., 1892, p. 423.

[1] Phil. Trans., Vol. 186, p. 570. With ethyl alcohol Worthington records a tension of 17 atmospheres. See Proc. R. Soc., Vol. l.

[2] Phil. Trans., pp. 563, 567.

[3] Proc. Roy. Irish Acad., Jan. 13, 1896, p. 767.

walls of the evaporating cells,' is therefore preferable. But Askenasy seems to me to put the matter more conveniently by using the term 'imbibition[1].' The force with which vegetable membranes, e. g. the thallus of Laminaria, absorb water, has been demonstrated by Reinke and others, and the existence of such a force is familiar to botanists.

Both Askenasy (loc. cit.) and Dixon and Joly[2] have pointed out that the force of imbibition, or the surface tension forces, as the case may be, can exert a tractional effect on the water in the tracheals, when the turgescence of the mesophyll has been destroyed. But Askenasy in his original paper (1895), Dixon in the January 1896 paper, and again Askenasy in his second paper (March, 1896) have also considered the imbibitional or surface tension forces in connexion with the turgescent cell. In his 1896 paper Dixon in fact gives up the view published in the Phil. Trans. and adopts the view given by Askenasy in his original paper, that the tractional force is supplied by the osmotic suck of the leaves. It must clearly be understood that this does not remove imbibition from the problem. It is one of the chief merits of Askenasy's work that he clearly sees and states the important relation between these forces[3]. The sun's heat causes the evaporation of the water with which the walls of the mesophyll cells are imbibed; this water is replaced by imbibition from the cell-sap. The concentration of the cell-sap so produced maintains the osmotic force of the cell, which again exerts suction on the water on the tracheals[4].

I have now given, in its simplest form, the modern theory of the rise of water. Apart from the main idea, it combines the points of several familiar views. Imbibition becomes a factor of paramount importance, though not in the way that Sachs employs it. The suspended threads of water remind us of Elfving's capillary theory, while the living-element factor is represented by the turgescent mesophyll cells.

Resistance.—It is not possible to discuss the question whether the tractional forces in the leaf are sufficient for the work imposed on them until we know what is the resistance to the passage of water through wood. For it is clear that the work done by the leaf includes

[1] Loc. cit., 1895, p. 10.

[2] Annals of Bot., Sept. 1895.

[3] Askenasy, 1895, p. 11.

[4] Sachs' Text Book, edit. iv, Eng. Tr., p. 679, describes evaporation taking place in the cell-wall, which makes good the loss by imbibition.

not only the lifting of a given column, but the overcoming of the resistance to its flow.

The resistance to the flow of the transpiration-current is in want of further investigation. Janse[1] has discussed the question, and points out (loc. cit. p. 36) that two kinds of resistance must be reckoned with. The first (which he calls statical) is illustrated by means of a cylinder of *Pinus* wood fixed to the short arm of a J-tube filled with water, when it was found that in five days the level of water in the long arm was only one mm. above that in the short arm[2]. That is to say, when time enough is given, the resistance is practically nothing. Janse has also investigated the resistance to the passage of water flowing through wood at the rate of an ordinary transpiration-current. His method seems to me open to criticism, but this is not the place to give my reasons. His experiments give a wide range of results. With *Pinus Strobus* a pressure of water equal to ten times the length of the wood was required to force water through at a pace equal to the transpiration-current. In *Ginkgo* the pressure was twenty-one times the length of the wood. Strasburger[3] has repeated Janse's experiment, and finds a column 'several times the length of the object' necessary. Nägeli[4] found that 760 mm. of mercury were needed to force water through fresh coniferous wood at the rate of $\frac{1}{21}$ mm. per second, i.e. at 180 mm. per hour. If we allow one metre per hour as a fair transpiration rate[5], we get a pressure of 5 atmospheres required to produce such a flow. To return to Janse's experiments: even if we assume that the resistance (expressed in water)=5 times the length, it is clear that with a tree 40 m. in height, the resistance of 20 atmospheres has to be overcome. This would not be a pressure greater than that which osmotic forces are able to exert, but when we come to a tree of 80 m. in height, and a resistance of 40 atmospheres, the thing becomes serious[6]. A great difficulty in the question of resistance is that the results hitherto obtained are (though here I speak doubtfully) much greater than those obtained by physicists for the resistance of water flowing in glass capillaries.

[1] Pringsheim's Jahrb., xviii. 1887, p. 1.

[2] Strasburger (Leitungsbahnen, p. 777) observed equilibrium established a good deal quicker.

[3] Leitungsbahnen, p. 779.

[4] Das Mikroskop, 2nd edit., p. 385.

[5] Sachs, Arbeiten, ii. p. 182.

[6] Schwendener's experiments, K. Preuss. Akad. 1886, p. 579, do not particularly bear on this question.

Until this discrepancy is explained, it is rash to argue from our present basis of knowledge [1].

Is the osmotic suck sufficient?—The osmotic force of a turgescent cell is usually measured by its power of producing hydrostatic pressure within the cell. Thus, De Vries [2] investigated the force necessary to extend a plasmolyzed shoot to its original length; Westermaier [3] the weight necessary to crush a tissue of given area; Pfeffer [4] the pressure exerted by growing roots; Krabbe [5] the pressure under which cambium is capable of maintaining its growth.

The figures obtained by these naturalists have a wide range; it may be said that the hydrostatic pressure varies between 3 and 20 atmospheres.

Another method is to ascertain the osmotic strength of the cell-sap in terms of a KNO_3 solution, and calculate the pressure which such a solution can produce. According to Pfeffer [6], 1 per cent. KNO_3 with artificial membrane gives a pressure of 176 c.m. = 2·3 atmospheres. De Vries [7] calculates that in a cell, a 0·1 equivalent solution (practically = 1 per cent.) gives a pressure of 3 atmospheres. We may therefore take it as between 2·5 and 3 atmospheres. Now, De Vries found that beetroot requires 6–7 per cent. KNO_3 to plasmolyze it; this would mean 15–21 atmospheres. I do not know what is the greatest pressure which has been estimated in this way. Probably Wieler's [8] estimate of the pressure in the developing medullary ray cells of *Pinus sylvestris* at 21 atmospheres is the highest. It is clear that investigation of the osmotic capacity of leaves for high trees is wanted, also investigations of the variation in osmotic power produced by varying resistances in the flow of the current. The experiments of Pfeffer and others [9] show that the osmotic strength of cell-sap is capable of great adaptation to circumstances—cells respond by

[1] It is possible that the rate of the ascending water is much less than is usually assumed. Thus Schwendener (K. Preuss. Akad. 1886, p. 584) calculates from an observation of v. Höhnel that the transpiration-current in the stem of a tall beech was only two metres per day.

[2] Untersuchungen über d. mechanischen Ursachen der Zellstreckung, 1877, p. 118.

[3] Deutsch. Bot. Ges. 1883, p. 382.

[4] Abh. k. Sächs. Ges. 1893.

[5] K. Akad. Berlin (Abhandlungen), pp. 57, 69, 1884.

[6] Pfeffer, Phys. i. p. 53.

[7] Pringsh. Jahrb. xiv. p. 527.

[8] Pringsh. Jahrb. xviii. p. 82.

[9] Pfeffer, Abhand. der k. Sächs. Ges. xx. p. 300; Eschenhagen, Untersuchungen aus d. Bot. Inst. z. Tübingen, 1889; Stange, Bot. Zeit. 1892.

increased turgescence to various stimuli. Whether they can respond sufficiently to account for the ascent of water is another question.

My own opinion is that the question of resistance to the flow of water is a difficulty which the authors of the modern theory have not sufficiently met. Unless it can be shown that the resistance to the flow of water in wood is less than that indicated by existing researches, we must face the fact that we do not at present know of osmotic forces which we can suppose capable of raising water to a greater height than 40 metres.

Continuity of the water in the tracheals.—The theory we are considering apparently requires that there shall be continuous columns of water from leaf to root, because a break in the column means a collapse of the machinery. This seems at first sight a fair assumption, though I doubt its complete correctness. It is in any case worthy of discussion. It has been constantly insisted on by Sachs and others that at the time of most active transpiration the vessels contain air, and not water. It is therefore a violent disturbance of our current views to believe in continuous columns of water.

For evidence on this point we are chiefly indebted to Strasburger. It is a remarkable fact that he should, without any theory to encourage such a view, have come to the conclusion that approximate continuity of water columns is a condition of primary importance, and that he should have made out the cognate fact that the whole of the *alburnum* need not be simultaneously occupied by a transpiration-current; parts of it may be so occupied, while parts of it are filled with air, and do not function as waterways. This is a valuable contribution to knowledge, and to the adherents of the new theory it is priceless; the very existence of their hypothesis may depend on it.

Strasburger's statements and reasoning are by no means accepted by every one; for instance, Schwendener refuses to take them seriously [1].

Strasburger has microscopically examined the condition of the tracheals as regards air [2]. He found in the Spruce Fir in July 'almost no air bubbles' in the wood of the current year, but air in considerable

[1] K. Preuss. Akad., 1891, p. 931.

[2] Leitungsbahnen, p. 683 et seq.; Russow in 1882 (Bot. Centr., Vol. xiii. 1883) observed similar facts in the distribution of water and air.

quantity in four-year-old wood. In the same month *Pinus Salzmanni* (*Laricio*) showed scattered bubbles in the spring wood of last year, and more in the autumn wood. In a Larch there were only very occasional bubbles in the two last years' wood. In the Silver Fir the current year's wood was practically free from air: the air increased in the inner rings. *Tsuga canadensis* had no air in this year's wood, only a little in last year's, and an increasing quantity in the older rings, the fifth being very rich in air. In February, *Pinus Strobus* had hardly any air in this year's wood, and the Silver Fir was all but free from it in the youngest ring. *Robinia* in July had the youngest wood almost air-free. *Ficus elastica* and *spuria*, various *Acacias*, and willows gave vessels not entirely free from air, but nearly so. He concludes[1] that the path of the transpiration-current is not absolutely free from air. The younger wood, which especially functions as the water-carrier, is the most free.

Dixon and Joly quote Strasburger's results, which they consider sufficiently favourable to their views. They rely, in addition, on the impermeability of wet cell-walls to air isolating the conduits in which air has appeared; and on the possibility that the air may be redissolved under root pressure[2], an idea well worth testing.

I think Strasburger's facts are not so favourable to their theory as these authors believe; in the same way it seems to me that Askenasy is rash in saying[3] that the tracheals in many cases contain continuous columns of water. It is true that this statement does not affect the validity of his general argument, since he faces the undoubted occurrence of air bubbles in many cases. This is undoubtedly necessary, and fortunately we can once more turn to the Leitungsbahnen. Strasburger states that he has seen water creep past the air-bubbles[4] in coniferous tracheids. The best evidence for this seems to be the fact mentioned[5] that the part of a single tracheid in front of an air bubble gets red with absorbed eosin, though the neighbouring tracheids are colourless. This clearly suggests the creeping round the bubble which Strasburger believes in. Schwendener[6] has been unable to confirm Strasburger's microscopic observations, and moreover denies the physical possibility of the phenomena. I am unable to judge of the

[1] Loc. cit., p. 688.
[2] Phil. Trans., p. 572.
[3] Verhand. naturhist. med. Vereins Heidelberg, 1895, p. 15.
[4] Leitungsbahnen, pp. 704, 709. See also Hist. Beitr. v. p. 76.
[5] Ibid., p. 79.
[6] Zur Kritik, &c., p. 921.

validity of Schwendener's theoretic objections, and must leave this point. It is a question of great importance whether it is possible that on the breaking of a column of water a film of water remains surrounding the air bubble, and capable of holding the two columns together. If this is impossible, we must suspend our judgment until we know more of the contents of the tracheals.

To sum up this part of the subject, we may believe that the tracheals in their youngest condition may contain water in continous columns, since the cambium cells from which they arise certainly contain fluid. But we know also that this condition is not absolutely maintained, since Strasburger has shown that the young wood contains air, though in small quantity. We must therefore believe either (1) that the transpiration-current is able to travel past the air bubbles, or (2) that tracheals partly filled with air may again become continuous waterways by solution of the air. If we adopt the first alternative, we must believe that the film of water between the bubble and the wall of the vessel is able to bear such a tensile stress that it can serve to link the column above with the column below the bubble. But this is analogous to trusting a rope so nearly cut through that only a few threads remain intact. With regard to the second alternative, we have at least indications from Strasburger's work that a tracheal, partly filled with air, does not necessarily remain permanently functionless (see Leitungsbahnen, p. 692).

The isolation of the tracheal.—There are a number of points connected with the structure and properties of wood which ought to be considered in relation to the modern theories. Want of space forbids my doing more than referring to two of them.

The resistance which the wetted cell-wall offers to the passage of undissolved air is a point on which many writers have laid stress. It is clear that on any theory of the movement of water in the tracheals it is essential that air should not filter into the waterway. This necessity is not, however, stronger in the case of the modern theories we are considering. The pressure tending to fill the tracheals with air from outside cannot be greater than atmospheric pressure, and since the wetted cell-walls of gymnospermous wood can resist the passage of air under a pressure of about an atmosphere [1], we need not fear criticism of the theory on this ground. The above remarks

[1] Leitungsbahnen, p. 722. Nägeli and Schwendener, Das Mikroskop, 2nd edit., p. 367, give 225 cm. of mercury.

seem, however, to be needed in face of the frequently recurring statement that wetwood membranes are impermeable to free air. Schwendener has some good remarks on this head[1].

Strasburger has called attention to the important subject of the localization or isolation of vessels or of certain lines of tracheids. When this is possible we may have one set of tracheals containing continuous water-columns, while neighbouring ones contain air at negative pressure[2]. This is especially important in connexion with the theory of Dixon and Joly and of Askenasy, since, if there were no such isolation, a functioning tracheal containing a continuous column of water would give up its water to one which was not functioning. In other words, the inactive tracheals would, by negative pressure, suck water from the active ones. In the coniferous trees the young wood is cut off by the absence of pits in the tangential walls[3] from free communication with the older wood, where air is more frequent.

In the same way the valve-like closure of the pits by the aspiration of the pit membrane comes to be a subject of much importance.

At present I merely wish to show by a couple of examples the necessity of a complete study of the minute structure of wood in relation to the modern theories. It is at least a hopeful fact for the authors of these views that we cannot point to anything in the anatomy of wood which is absolutely inconsistent with their views. Finally, with regard to the question at large: Whether we are friends or opponents of the new theory, the broad facts remain that water has the power of resisting tensile stress, and that this fact must henceforth be a factor in the problem. There are difficulties in the way of our authors' theory, but it is especially deserving of notice that many of these difficulties are equally serious in the case of any theory which excludes the help of the living elements of the wood, and assumes a flow of water in the tracheals. The authors have not only suggested a *vera causa*, but have done so without multiplying difficulties. There is therefore a distinct balance in their favour.

Huxley, quoting from Goethe, makes use of the expression *thätige Skepsis*. It is a frame of mind highly appropriate to us in the present juncture if we interpret it to mean a state of doubt whose fruit is activity, and if we translate activity by experiment.

[1] Zur Kritik, p. 943.

[2] See Histolog. Beiträge, v. p. 87.

[3] Strasburger discusses, in this connexion, the existence of tangential pits in the autumnal wood: see Leitungsbahnen, p. 713.

PROFESSOR VINES said:—

It must, I think, have come as a surprise to many of the general public here present, that we are not yet in possession of a coherent theory as to the mode in which water is conveyed to the leaves from the roots of trees. It is almost incredible that though this problem has been the subject of research for more than a century, it still remains, as Mr. Darwin has pointed out, without its solution. But after the admirable account of the history of the subject to which we have been listening, there is no occasion for me to say anything about the past. My object in taking part in this discussion is to give some account of observations which I have been recently making, in the endeavour to carry the subject a step further onwards.

The particular feature of the problem which seemed to me to specially call for investigation is what is termed the *suction-force* of branches. I may explain what I mean by *suction-force* by referring to an experiment which has, I feel sure, been performed by every one in this room. When a gathered flower is placed in a glass of water, it sucks up a certain amount of the liquid; and it does so with a certain force, as can be proved by appropriate means: this force is the suction-force of the flower. Many have already measured the suction-force of various branches, &c., but it does not appear to me to have been done so systematically as to afford any important body of evidence on the subject. With this end in view, I have devised an apparatus[1] which combines simplicity with sensitiveness. I may briefly describe it as a system of tubes, partly glass, partly thick india-rubber pressure-tubing, completely filled with water, connected air-tight on the one hand with the branch under experiment, and on the other with a Bourdon's vacuum-gauge: the readings on the dial of the gauge are in inches of mercury, and indicate the suction-force exerted by the branch. I thought at first that I had succeeded in devising an apparatus which possessed the special advantage that it indicated suction-force without the intervention of atmospheric pressure, and that therefore my results were different in kind from those of previous observers. I am glad to have this opportunity of distinctly stating that I was mistaken in this conclusion: as a matter of fact, the atmospheric pressure does contribute to the readings given on the dial. I was misled by the fact that the readings of the gauge are due to the withdrawal of very small quantities of water; so small,

[1] Described and figured in Annals of Botany, Vol. x, No. 39, September, 1896.

that I was led to believe that the branch did not absorb any water at all from the apparatus, but merely exerted a tensile stress upon it.

I am not now going to inflict upon you an account of the observations which I have already published; I will merely state the conclusions to which they led. I was struck with the relatively high degree of suction-force which can be developed by a branch after its leaves have been removed; and this led to the consideration that it is important to investigate the suction-force of stems quite independently of the leaves in the first instance. I cannot but think that hitherto the problem has been obscured, rather than otherwise, by introducing considerations based on the action, real or supposed, of the leaves in the development of the suction-force of branches. I would urge that it is of primary importance to study the stem, and to ascertain what it is capable of effecting by itself.

In the second place, I was struck with a fact upon which Mr. Darwin has laid stress, namely that the suction-force of a branch is not essentially dependent upon the life of the branch. I found that if a branch be killed by the injection of sulphate of copper, it still can develop a very considerable suction-force. Now in a branch thus injected, the normal conditions of osmosis must be altogether upset, and yet water reaches the evaporating surface: hence osmosis does not appear to be an essential condition to the conduction.

I now pass on to observations which I have since made. The logical outcome of the preceding considerations was to institute observations on dead leafless branches. For this purpose I used dead hazel-branches (which had served in the garden as pea-sticks during the summer); branches, that is, which, if I may so say, had died a natural death. I found that, in order to make satisfactory experiments with these sticks, it is necessary to previously inject them with water, and to cover all exposed cut surfaces with melted paraffin. In illustration of the results attained, I may mention that the best result was a suction-force of 19½ inches of mercury developed by a piece of stick 18 inches long. This establishes the fact that a very considerable suction-force is developed by a branch independently of leaves or of life; and this is a fact which demands complete investigation. The problem of the travelling of the transpiration-current is here presented in its simplest form. We have a small piece of dead branch, without leaves, without osmosis, developing a force which would probably suffice to raise a column of water to a height

of about twenty feet. The problem is a purel distinguished from a physiological—one. What ar forces active in this stick? When we thoroughly u physics of a piece of dead stick, it will be easy t physiology of the transpiration-current in a living tre then.

The suction-force developed by pieces of stick is ver I must admit that I have not yet succeeded so far as control or account for the variations which I have illustration I may cite the following experiments mad lengths of the same piece of stick, the duration of th being nearly equal:—

Stick = 36	in. long:	max. suction-force	= 16½	in.:	dur
,, = 18	,,	:	,,	,,	= 19½	,, :
,, = 9	,,	:	,,	,,	= 16½	,, :
,, = 4½	,,	:	,,	,,	= 6½	,, :

I am quite unable to explain these results; but no susceptible of some simple physical explanation— resistance being taken into account—for which the d present forthcoming.

It is a matter of congratulation to botanists that the transpiration-current is again attracting the attention The firstfruits of this renewed interest are already in the form of the papers published by Messrs. Dixon and shall have the privilege, as I understand, of hearing m work from one of the authors this morning. It is not attempt a criticism of the theory of the transpiration they propound: but I would venture to say this, that t of which I have given you some account lead me to a with Mr. Darwin in the opinion that these authors d attach sufficient importance to the part played by the i of the cell-walls. Messrs. Dixon and Joly assert, ind transpiring leaf the operative force is given by the meni in the membranous *réseau* of the evaporating cell-walls the physical properties of the cell-wall are of so much these superficial cells, they must be equally important more deeply placed cells; for these too are receiving water like the superficial cells, though not by evapo

these observers prove that water can travel in the cell-walls of branches the wood of which has had its lumina injected with paraffin, their paper fails to suggest what relation, if any, this property of the cell-walls bears to the upward passage of water in the lumina under normal conditions. It seems to me that it may be fairly demanded of any proposed theory on this subject, that it should explain the relation between the characteristic physical properties of the walls of the wood-vessels and the passage of water through their lumina, more especially with reference to the question as to whether or not imbibed cell-wall may be regarded as maintaining continuity of liquid between, say, the upper and lower portions of a tracheide, the central portion of which is occupied by a bubble of air. However, it is to be hoped that some light will be thrown on this point in the course of Professor Joly's contribution to this discussion.

Professor Joly read the following paper:—

I deem it necessary to preface any contribution which Mr. Dixon and I may make to this discussion by some remarks of the nature of claims to priority. Claims to priority are distasteful writing and distasteful reading, and I will hurry over them. I, at the same time, ask your indulgence as one acting under obligations which may not be shirked.

Our paper On the Ascent of Sap[1] was received by the Royal Society on October 16, 1894, and read November 15, 1894. It was written before the Abstract[2], which appeared November 15, 1894.

A considerable portion of our paper was excised by desire of the Publication Committee of the Royal Society; and after many delays it appeared in the Philosophical Transactions with the note 'Revised April 20, 1895,' at the head of it.

A restatement of the principal matter in the Abstract from the pen of Professor Askenasy[3], appeared February 12, 1895; about four months after our complete paper was received by the Royal Society. Professor Askenasy's paper is prefaced by the remark that its publication was hastened by the appearance of our Abstract. The writer admits he had made no researches of his own upon the subject, and accounts for his inactivity by the statement that he had considered

[1] Philosophical Transactions, Vol. 186 (1895) B.
[2] On the Ascent of Sap (Abstract), Proc. R. S., Vol. lvii, No. 340, Jan. 1895.
[3] Ueber das Saftsteigen, Heidelberg, 1895.

Strasburger's experiments to present sufficient data i
solve the problem. I think it will presently be se
view Professor Askenasy was mistaken; meanwhile
that Professor Askenasy himself appears to have char
upon this point, as in his second paper[1] (April, 18
before us actively engaged upon experiments similar
the same teaching as, some of our earlier ones.

The leading idea of our theory suggested itself to us
in our investigation. The following difficulties nat
themselves:—

(1) Is water containing air in solution and under
must obtain in high trees (according to this hypothesis

(2) Will this stability also exist in presence of wette

(3) Is the leaf capable of exerting such a lifting for
to raise the column of water in high trees?

Of these questions, the first had previously only be
quately considered, observers treating the presence
as detrimental to success in experiments on the beha
in tension; the second, so far as I am aware, had never
considered, nor had the third received any attention.
are obviously inseparably connected with the adm
theory, and admit of being answered only by expe
consideration occupy a large part of our paper as
(over and above experimental work relating to the
other hypotheses, most of which was excised from o
for many months of careful investigation.

We, on our part, conceived of our theory quite
unaware of any suggestion even remotely resemblin
tion of the cause of the ascent of sap in high
connexion I gladly repeat our expression of indeb
fessor Fitz-Gerald for many valuable suggestions i
our investigations.

Lest I be misunderstood I will state definitely that
I have no cause of complaint whatever against Prof
It is, indeed, only fair to quote his full acknowledg
lines from his second paper will suffice:—'As I have
first paper, Dixon and Joly are the first who have cle

[1] Beiträge zur Erklärung der Saftsteigens, Heidelbe

and formulated the significance of the cohesion of water in the ascent of sap.'

A further matter for consideration suggested itself over and above those enumerated; a collateral question, it is true, but one nevertheless of great interest and importance:—What is the *nature* of the suction-force in the leaf?

Our earlier ideas led us to the view that capillary actions and the phenomena of evaporation were mainly concerned with the upholding and raising of the sap, as will be more fully gone into further on. I gather from Professor Askenasy's papers that, in so far as we regard capillary phenomena (or as he prefers to call them 'imbibition' phenomena) as upholding the sap, we were then in accord with his present views. He claims priority for these views, however, giving as his reason that they are not so definitely stated in our Abstract as in our full paper, and, perhaps misled by the term 'revised' occurring at the head of our complete paper, insinuates that we borrowed them, in their more pronounced statement, from his paper of February, 1895. (See pp. 19, 20, Beiträge zur Erklärung des Saftsteigens.)

This accusation is easily disposed of. I quote (translating) in full from Professor Askenasy's paper:—

'I come now to the imbibition of the cell-wall, the other[1] chief factor in the ascent of sap, from which the true (eigentliche) sucking action proceeds. The imbibition force of the cell-wall is in fact the so long vainly sought for source of the suction-force concerned in the ascent of sap in the plant. I believe myself to be the first to point out clearly the importance of imbibition in the ascent of sap, since Dixon and Joly speak of it in their first communication in a quite uncertain manner:—"Whether the draught upon the sap established at the leaf during transpiration be regarded as purely capillary or not, their experiments lead the authors to believe that it alone is adequate to effect the elevation by direct tension of the sap in tall trees."

'In the complete communication which appeared after my first work it runs for the first time thus:—"The meniscuses are formed in the membranous *réseau* of the evaporating cell-walls, while the columns of liquid supplying the evaporation loss exist in the functioning conduits."'

It will be seen that the quotation from our complete paper is advanced

[1] Refers to the stability of a liquid in tension and containing gas in solution.

by Professor Askenasy as representing his views. type-written copy of our original manuscript, and th the possession of the Royal Society and doubtless Reference to either will show that the sentence Askenasy quotes as representing his views on the r our original manuscript, and was published from it wi a syllable. The priority, for what is, at most, but a sh is thus given away by his own decision.

After what I have quoted from Professor Askenasy a force of the cell-wall being 'the so long vainly soug the suction-force concerned in the ascent of sap in t perhaps appear surprising that Mr. Darwin interprets nasy as advancing the view 'that the tractional force is osmotic suck of the leaves'; and, taking this view, d paper of 1896[1] as a reversion by Dixon to Professor A of 1895. The quotation from Professor Askenasy wh above is, indeed, from his second paper; but in his 18 Professor Askenasy asking leave to modify a stateme forces are responsible, when he recalls how in dead bition force alone can accomplish this, and procee a length of several pages experimental evidence in view. Professor Askenasy does indeed refer to o active in transferring the sap from the bundles into leaf; but we ourselves, in our original paper, suggest this action occurs. In dwelling upon the great import pressure, Dixon advances alike beyond the views c original paper and those put forth by Professor Ask be seen presently, for the explanation of the active lifti leaf, we have not to go beyond our original stateme referable to the phenomena of evaporation in the lea knowledge definitely extends[2].

[1] Note on the Rôle of Osmosis in Transpiration, Proc. Roy January, 1896.

[2] 'Although osmotic actions may be concerned with the ev of the leaf, i. e. in the transference of water into the protoplas it is very probable that surface-tension forces developed upon th coming in direct contact with air diffusion currents are respons the tensile forces displayed by the leaf. The fact that transp accelerated by direct sunshine, but even more influenced by supports the view that evolution of vapour at the leaf obeys

The nature of each phenomenon concerned must be clearly kept in mind with regard to whether it is of a static or of a kinetic nature; whether merely a sustaining action or one actually giving rise to motion. It will tend to clearness to cite from Dixon's paper of 1896 (on the 'Rôle of Osmosis in Transpiration,' pp. 773–74) the model or system which he imagines as diagrammatically representing the rôle of osmosis in transpiration.

A porous pot rendered semi-permeable is attached, water-tight, to the upper extremity of a glass tube. Pot and tube are filled with water, and the lower end of the tube opens into a vessel of water. Above, a bladder or other semi-permeable membrane is supposed to inclose the porous pot loosely, the mouth being closed by a water-tight binding around the tube below the pot. We suppose an aqueous solution of potassium nitrate in the space between the bladder and pot. We call the region inside the pot A; the space occupied by the osmotic solution B; the external atmosphere C. The following actions I will now suppose to occur, and may be described as the teaching of the model:—

(1) If B is at first not quite filled with liquid, the osmotic attraction upon the water in A, or, according to recent views upon the nature of osmotic action, the osmotic pressure, will gradually suffice to quite fill it. Water will consequently ascend the tube. In this operation the membrane is at first loose and, of course, any force located in it is clearly unable to exert any independent lifting pull upon the water in A; nevertheless water ascends. This is the case of the revival of a drooping leaf in which upon an adequate supply of water turgescence is re-established.

(2) The bladder becomes tense under increasing pressure in the space B.

I will assume now that a capillary or imbibition attraction of the membrane alone acts, and suffices to keep the external surface of the membrane moist. Evaporation now progresses upon the surface, and the current of water upwards in the tube is maintained. What

of evaporation from a moist surface' (Phil. Trans., Vol. 186 B, p. 567). The paragraph which follows refers to the 'sorting demon' nature of evaporation and the inflow of thermal energy at the evaporating surface in support of the view that to evaporation phenomena the elevation of the sap is to be ascribed. The same views are urged throughout the Abstract, and we admit that 'other physiological phenomena' may act (p. 5).

rôle does the imbibition or capillary attraction of the in this system? Once the steady state of supply a regard to evaporation loss is established, it can pla The attraction upon the water as much acts to reta to elevate the sap. It is a purely static and undirecte no work. Without any more accurate knowledge of i than we at present possess we are safe in saying this

In this system, therefore, we have an osmotic pre membrane tense and the cell filled with water (and w present, doing no more); an imbibition force locat brane keeping the membrane permeated with wate the boundary of the system evaporation progressing. a system the only kinetic action effective in actuall —in fact the only directed action—is that derived energy of those water molecules which escape from surface or meniscuses; their loss being made go attractions and diffusion forces acting within the li the actual lifting forces; the work done in lifting t gravity and viscous resistance being but small c normal work of evaporation. The energy expende finally restored by the inflow of heat at the evaporati

Again, from another point of view, the movemen the osmotic cell may be broadly referred to the diffe vapour pressure in the conduits and in the intercellul

Of the two static forces—osmosis and imbibition single out one as especially *the* upholding force? pressure, indeed, in the drooping leaf actually playi and functioning as a directed force. In the steady assumed it to do so any longer. Again, the im located in a membrane owing its rigidity entirely, i osmotic pressure. Are we justified in selecting the as the long sought for source of the suction-force elevation of the sap? I see no reason for doing so.

But this does not exhaust the question; a matter remains over. The actions enumerated above do n suffice for the phenomena concerned with the asce living leaf. It happens that it is possible to test forces concerned independently of the other—that o pendently of osmosis. If a leafless stick of some co

is injected with water and left standing in a vertical position, the lower end dipping in water, it is found, after some time, to dry back from the upper cut surface. Here imbibition of the cell-wall shows itself inadequate to draw up water against gravity and the viscous resistance of the liquid. How then is it able to functionate in the leaf? Again, a cut branch fed with a liquid injurious to osmotic action shows, first, diminished transpiration, ending in complete drying up of the leaf. The same effect may be brought about by exposing the leaf to a temperature sufficient to kill the protoplasm: a 'damp heat' of 80° C. Lastly, we must consider that in some cases individual cells show themselves capable of effecting an actual extrusion of water by internal actions. The unicellular Fungus, *Mucor*, shows this in a marked degree. *Pilobolus* and *Phycomyces* may also be mentioned. The phenomenon of the extrusion of water by certain cells at the base of the petiole of the sensitive plant (*Mimosa pudica*), when responding to stimulation, is probably another case.

All this suggests that the actions progressing in the turgescent cells actually assist the extrusion of water. Indeed, we may go further and state more positively that the inadequacy of imbibition when unaided to maintain the moisture at the surface of the cell-wall on the one hand, and the evidence for the existence of extrusive forces in the turgescent cell on the other, at once forbid us to close the question as settled, and suggest where to look for the answer.

But what is the nature of the extrusive forces?

It would be a matter of profound interest if it turned out that 'vital' actions entered into this question. Or, if 'vital' processes in the primordial utricle are not responsible, can we refer the action to osmotic pressure alone? If we do so, we certainly find ourselves in the difficulty of ascribing to this force the opposite effects of securing the entrance of the sap into the cell against the tension in the conduits, and its subsequent expulsion through the cell-wall.

While this appears hardly admissible it is very certain that the osmotic pressure in the cell is effective in largely promoting the evaporation in so far as its action upon the flexible membrane results in the distension of this. This distension may amount to as much as from 10 per cent. to 20 per cent.[1] We may imagine the elastic shrinkage of the wall and its reduction in volume under the tensile

[1] Strasburger, Lehrbuch der Botanik, p. 138.

abstraction of water, when unsupported by osmo
largely increase its resistance to the passage of water
time diminish its effectiveness as an evaporating area,
of the diminution of this area, but also because
fineness of grain and consequently increased capill
The stretching of the wall by the osmotic pressure, o
increases both its permeability and its evaporating eff
result of osmotic pressure in the cell permits us to re
maintaining the water upon the outer surface of th
against the elastic force of the cell-wall and the tensil
When the steady state is reached, it is however none the

While something of what I have already said is,
bution to this discussion, and over and above perso
with pleasure to the unalloyed scientific interest
mentioned in Mr. Darwin's paper.

Mr. Darwin points out the want there is for obs
osmotic pressure of the cells of leaves of high t
account for the high tractive forces required to e
against gravity, and the viscous resistance of the asc
liquid. I am glad to say that Dixon has already
towards supplying the want and is actively engage
matter further.

In a paper of his read June 8, 1896, before the Roy
he gives an account of his preliminary researches.
yet published: I have the proof sheets here, however

The mode of experimenting is already described
(pp. 564–66) and need not be re-described. The
on the assumption that a collapse of the leaf unde
gaseous pressure will indicate the limit of the osm
the turgescent cell; the water is then supposed to
into the conduits and ejected from the cut end of th
from the nature of the arrangements, is not expo
gaseous pressure.

In Dixon's experiments it is important to note,
at which what we may call the *saturated* osmotic cel
water into the conduits; and secondly, the higher
the leaves begin to collapse and lose their turgescenc

Similarly to the manner described in our paper in
Transactions, the first phenomenon is observed b

intervals of time and at different pressures, of the weight of a small phial of water surrounding the cut stem of a branch which, previous to experiment, has been left standing for some time in water.

The pressure first giving rise to the ejection of water under these circumstances assigns a limit to the functioning of the cell as one holding the maximum quantity of water which its voluminar distension admits.

The second phenomenon is observed directly in the curling up of the leaves, which roll slowly inwards from the edges and simultaneously droop upon the petiole. By relieving the pressure the leaf will in many cases recover its original form in the course of a few minutes. It may, in fact, be caused to curl up slowly and open out repeatedly like a Bourdon tube. The pressure giving rise to crumpling is that which assigns a limit to the ultimate tractive force of the cell and to its functioning as a healthy organ.

A striking fact is revealed in the course of these experiments:—The use of carbon dioxide as the agent for producing the pressure gives a markedly lower result than the use of air. In fact the limits of resistance fall to about one-third or one-fourth of their value.

In the case of *Cytisus Laburnum* a pressure of between 6 and 8 atmospheres of carbon dioxide will cause the crumpling of the older leaves, although the younger ones will still remain turgescent: in fact 16 atmospheres of carbon dioxide were attained without crumpling of the younger leaves being observed. First at 26·6 atmospheres of *air* the older leaves of *Cytisus* began to curl up. At 20 atmospheres of air the saturation limit of the osmotic force was reached.

In the case of *Tilia americana* a pressure of between 7 and 8 atmospheres of carbon dioxide gave slight collapse, while, according to Dixon's most recent experiments, a pressure of 20 atmospheres of air did not produce collapse. The saturation limit for this tree was reached, when carbon dioxide was used, at 4 atmospheres. With the use of air it was reached at 15 atmospheres upon a branch which had been already experimented with. A prior observation upon this branch gave no loss of water at 15 atmospheres of air.

In the case of *Helianthus multiflorus* 20 atmospheres of air produced collapse, the rolling up and flagging of the leaf being beautifully marked. At 13 atmospheres it held out. The osmotic limit of resistance may therefore be taken between 13 and 20 atmospheres in this plant.

The experiments are still in progress; but we have sufficient in these results to indicate an enormous potential osmotic tractive force in the case of tall trees such as *Tilia*. In this case the limit of this force *was not reached* at 20 atmospheres.

Following Mr. Darwin in assuming that the resistance to motion at the transpiration velocity is given by a hydrostatic pressure equivalent to five times the length of the branch, this minor limit of osmotic pull suffices for a lime tree 100 feet high. The limit of height recorded for *Tilia americana* is sixty feet. In the case of the Laburnum, this tree might, so far as Dixon's results indicate, flourish to a height of 130 feet. Its recorded limits are between thirty and forty feet. Similarly the Sunflower might attain a height of eighty feet or thereabout. It appears therefore as if it was not osmotic conditions which limited the heights of trees; although the limit, in the case of the lower growing plant, is the lowest observed.

I may mention here that, so far, conditions of sunshine or shade have not been observed to notably alter the results. If not freely supplied with water, however, the branches might indicate differently. Dixon hopes yet to make an observation upon the branch *in situ*.

The following table shows the weights of water transpired by similar branches exposed to like conditions, as far as possible, save that one was immersed in carbon dioxide and the other surrounded with air. The pressure on each was the normal atmospheric pressure:—

Helianthus multiflorus.

Normal pressure.

Surrounding medium.	No. of leaves.	Time.	Weight transpired.
A. Air.	10	90 mins.	0·302
a. Co_2.	10	90 mins.	0·257
B. Air.	10	60 mins.	0·372
b. Co_2.	10	60 mins.	0·108
B. Air.	10	180 mins.	0·598
b. Co_2.	10	180 mins.	0·236
B. Air.	10	24 hrs.	2·862
b. Co_2.	10	24 hrs.	1·979
C. Air.	12	24 hrs.	5·230
c. Co_2.	12	24 hrs.	2·240

In this table the letters prefixed indicate the identity of a branch used a second or third time. The branches B and b were, it will be seen, used in three succeeding experiments.

As regards the condition of the leaves at the conclusion of the experiments, it is to be remarked that but little difference was to be detected in the degree of flagging exhibited by specimens which had transpired in air and in carbon dioxide; on the whole the latter were the fresher.

I do not think that any one considering these results can fail to be struck by the inference that the great differences in the rate of transpiration may in a great measure be due to a lowering of vital activity in the one case. But of course, pending further and more varied experiments on this complex matter, the question hardly admits of profitable discussion.

The question has been raised as to what may happen in a tracheid containing air bubbles. I may say that our views on this subject are as follows:—

There will be a critical size for the bubble corresponding to the prevailing tensile stress. If below this dimension, the surface-tension force of the bubble will be adequate to cope with the tensile stress of the sap and the vapour and air pressure within it. It will then retain its minute dimensions and no more interfere with the circulation of water than would an equally minute solid body. If, on the other hand, it is of a dimension incapable of withstanding the expanding forces enumerated, it will yield and expand so as to occupy all but the sharp corners of the conduit or deep depressions upon the walls. Except within the cell-wall, we think that, under these circumstances, there will be no motion of water past the bubble in the conduit under consideration. Increase of tension will not tend to further a free current past the bubble, rather it will cause this to expand still further and carry the gaseous space deeper into the surface of the cell-wall.

In this connexion I may observe that the manner in which a hydrostatic tension is transmitted through the column of sap in the tree is sometimes a subject of some misconception, and many appear to regard our theory as postulating the existence of hanging thread-like filaments of water in tension, which must be supposed extending in continuous lengths from leaves to root in order to be effective. Hence, it is argued, the presence of a bubble anywhere in the path of one of these threads, by interrupting its continuity, destroys its

effectiveness, and the entire transpiration current is re
section throughout its length by the absence of one of
filaments. Such a view greatly exaggerates the effect
of free gas here and there in the lumina. It should
that the vessels and tracheides are not merely laid e
intercommunication confined to the conduit above
that where a lumen is occupied by a bubble the path
from the conduit beneath, for example, is simply d
adjoining conduit. Thus by the presence of an i
transpiration current experiences an opposition w
somewhat in the manner in which a river evades th
a stone in its bed. Or, if the idle lumina are numerous,
effect might be compared to that of sand opposing th
water. The hydrostatic tension is transmitted
directions, the lumina communicating as they do throu
membranes of the pits.

The rarity of the occurrence of a liquid in tensi
know if hydrostatic tension has been detected an
nature) almost justifies the misconception which ascribe
to hydrostatic tension and hydrostatic pressure. Esse
are the same, substituting for the idea of a pull, the ide

I would like to say a word as to some views which
commend themselves regarding the nature of the root-
drawing water from the soil. The tensile stress tra
root has ultimately the effect, we may say, of dryi
surface. We suggested this in our first paper. The
the same power of condensing water as dry oatmeal, p
would have if buried in damp earth; not necessarily
contact with damp particles, but by absorption of a
As a matter of fact the aqueous film which overlies so
immobile that it is hardly conceivable that a root-hair
a particle of silica, for example, could get more w
immediately adjoined its point of contact. The qu
taken up by trees appears to be often so large, even
that this older view of the phenomenon would we think
Of course a great deal is taken up in the liquid
necessary salts thus brought into the plant. Prof
seems to misconceive our ideas on this matter[1]. Tak

[1] Beiträge, p. 5, 1896.

the root-action, we can regard the whole phenomenon of the ascent of sap to depend broadly upon a difference of vapour pressure in the atmosphere surrounding the roots and in the atmosphere surrounding the leaves [1].

In reference to the doubt suggested by Mr. Darwin (p. 641) as to the re-solution of air by root-pressure sap, we would say that the *onus probandi* would rather lie with those who question such action. The root-sap contains only a small quantity of dissolved air, and, except the laws of absorption of gasses by liquids differ inside the tree from elsewhere in the world, we expect that it will be willing to take up more. Here too we may recall what Strasburger quotes [2] from Volkens and Vesque as to the appearance and disappearance of air bubbles in the conduits of herbaceous plants.

In regard to the general considerations of the stable properties of a dust-free liquid under tensile stress, it is interesting to notice the analogy with the behaviour of a dust-free vapour under pressure. In the first case an enormous stress is required to initiate a bubble of vapour, for the surface tension of this bubble will be the greater proportionately as its radius of curvature is smaller. In fact without actual fracture of the liquid, only a dry particle (exposing already a free surface of appreciable radius of curvature) can initiate the bubble. In the case of the vapour it requires a high pressure to initiate the precipitation or formation of drops, because the vapour pressure of these will be the greater as the radius of curvature is smaller. Here, too, we require a particle to initiate a wet surface of appreciably great radius of curvature. The increased vapour pressure at sharply convex surfaces may not be without influence in the evaporation at the surface of the minute mesophyll-cells, when these present a wetted surface to the intercellular space. I may remind you, in connexion with these remarks, of Aitkin's experiments on the counting of dust particles, and Maxwell's remarks on how small drops of rain are unstable in the presence of big ones [3].

Reviewing the whole matter in a few words:—

We have in the tracheal system of the plant a water-way which is freely open to water-movement, while closed to the movement of free gas. Every bordered pit is an open door to the sap and a closed

[1] Ascent of Sap, Phil. Trans., pp. 574-75.
[2] Leitungsbahnen, pp. 696-97.
[3] See Tait's Properties of Matter, Chapter on Capillarity.

one to the gas bubble, and one which locks and bars itself against the exit of an imprisoned bubble. In a word, it is a structure semi-permeable towards matter in its three states, the solid, the liquid, and the gaseous. Taking the nature of this structure into account; the observations on the swamped condition of a great part of it; the experimentally proved fact that water containing air and in presence of wet wood is stable under high tension; and that the phenomena occurring in the leaf are capable of giving rise to the necessary traction, it is not too much to say that one who questions the existence of tensile stress in the sap (when root pressure is inactive), because it has not been directly demonstrated, is in the same position as one who questions the existence of pressure in the deepest part of the ocean because observation there had never demonstrated it.

I may perhaps be permitted to remark more clearly here—in view of a misconception referred to early in this paper—that the mention of revision at the head of our complete paper in the Phil. Trans., refers to no change of opinion or modification of views expressed in our original manuscript. The term refers solely to excisions.

In conclusion I desire to express how cordially Mr. Dixon and I concur in Mr. Darwin's remarks upon the work of Strasburger. Whatever the fate of our hypothesis—and the clearest evidence may deceive; the strongest convictions err—*his* name must ultimately be for ever associated with the true theory of the ascent of sap.

Professor G. F. FitzGerald, in continuing the discussion, spoke of the difficulty of drawing distinctions between the physical nature of 'imbibition' and capillary forces; and the risk the use of the first term involved of substituting the word for the idea.

He did not consider that the supporters of Messrs. Dixon and Joly's theory required necessarily to prove the existence of tractive forces in the leaf equivalent to a hydrostatic head five times the height of the tree, in order to meet the viscous resistance of the sap. The estimate of the viscous resistance to be accounted for must be based on the velocity of the transpiration current in the higher parts of the tree under normal conditions of supply; and this, probably, was not ascertained. The actual movement of water in the higher parts of the tree might be very slow under normal conditions, and hence the viscous resistance very small.

The principles determining the permeability of a septum by the

medium in which it was immersed might be illustrated by the cases of a wet and dry fabric; the first freely permeable to water, the second freely permeable to air. Capillary resistance destroys the free permeability when it is sought to pass through the fabric a medium differing in physical properties from that already contained in the openings or meshes of the fabric. The fact that the wet fabric was not permeable by air illustrated the properties of the cell-wall in retaining a bubble developed within the lumen.

After some remarks from PROFESSOR MARSHALL WARD, the discussion closed.